fully available

BEST AVAILABLE COPY

(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) EP 0 570 357 B1

(12) EUROPEAN PATENT SPECIFICATION

(45) Date of publication and mention of the grant of the patent:
25.06.1997 Bulletin 1997/26

(21) Application number: 93890100.6

(22) Date of filing: 13.05.1993

(51) Int Cl.[6]: C12N 15/49, C12N 15/62, A61K 39/21, G01N 33/569, A61K 39/295, A61K 39/145, A61K 39/29, C07K 14/16, C07K 14/02

(54) Peptides that induce antibodies which neutralize genetically divergent HIV-1 isolates

Peptide, die Antikörper induzieren, die genetisch divergierende HIV-1 Isolationen neutralisieren

Peptides induisant des anticorps, qui neutralisent des isolées d'HIV-1 qui divergent génétiquement

(84) Designated Contracting States:
BE CH DE DK ES FR GB IE IT LI NL PT SE

(30) Priority: 14.05.1992 AT 987/92
29.08.1992 US 932787

(43) Date of publication of application:
18.11.1993 Bulletin 1993/46

(73) Proprietor: Polymun Scientific Immunbiologische Forschung GmbH
1190 Wien (AT)

(72) Inventors:
- Katinger, Hermann
A-1190 Vienna (AT)
- Rüker, Florian
A-1170 Vienna (AT)
- Himmler, Gottfried
A-1180 Vienna (AT)
- Muster, Thomas
A-1180 Vienna (AT)
- Purtscher, Martin
A-1180 Vienna (AT)
- Mailwald, Georg
A-1040 Vienna (AT)
- Steindl, Franz
A-1160 Vienna (AT)
- Trkola, Alexandra
A-1232 Vienna (AT)

(74) Representative: Büchel, Kurt F., Dr.
Patentbüro Büchel & Partner AG
Letzanaweg 25-27
9495 Triesen (LI)

(56) References cited:
- 8 TH INTERNATIONAL CONFERENCE ON AIDS JULY 19-24 1992 vol. 8, no. 2 , AMSTERDAM, NL M.PURTSCHER ET AL. 'Human monoclonal antibodies against HIV-1 in a longterm in vitro neutrelization study; pA73 abstract no. PoA 2422'
- AIDS RESEARCH AND HUMAN RETROVIRUSES vol. 6, no. 3 , March 1990 V.TEEUWSEN ET AL. 'Production and characterization of a human monoclonal antibody, reactive with a conserved epitope on gp41 of Human Immunodeficiency Virus type I'
- PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA. vol. 89 , January 1992 , WASHINGTON US pages 461 - 465 P.BROLIDEN ET AL. 'Identification of human neutralization-inducing regions of the human immunodeficiency virus type 1 envelope glycoproteins'
- FEBS LETTERS. vol. 233, no. 2 , June 1988 , AMSTERDAM NL pages 393 - 396 D.MCPHEE ET AL. 'Recognition of envelope and tat protein synthetic peptide analogs by HIV positive sera or plasma'
- Vaccines'92 191-195;A.Buchacher et al.

Printed by Jouve, 75001 PARIS (FR)

EP 0 570 357 B1

Description

This invention refers to peptides that induce antibodies which neutralize genetically divergent HIV-1 isolates. These peptides are applied with an adjuvant, as recombinant fusion proteins, chemically coupled to carrier molecules, as recombinant chimerical viruses or as recombinant antibodies. In addition, the stage of infection can be determined and the progression of the infection can be predicted with these peptides.

INTRODUCTION

The acquired immunodeficiency syndrome (AIDS) is the late stage clinical manifestation of long term persistent infection with human immunodeficiency virus type 1 (HIV-1). Immune responses directed against the virus and against virus-infected cells during the persistent infection usually fail to mediate resolution to the infection. A possibility to elicit an immune response that can prevent the establishment of a persistent infection or that can prevent the progression to AIDS are vaccines. Most vaccine strategies against HIV-1 are directed against the surface glycoprotein gp160 which is made up of gp120 and gp41 and is responsible for virus binding to the cellular receptor CD4 and fusion activity.

However, in context with gp160 several phenomenons that argue against the use of whole gp160 or gp120 as an immunogen were observed. *In vitro* experiments showed, that synergism between HIV-1 gp120 and gp120-specific antibodies block human T cell activation (1). This result supports the hypothesis, that also *in vivo* the humoral immune response against gp120 of HIV-1 suppresses T-cell activation and might be one reason for immunodeficiency. The proposed mechanism for this phenomenon is cross-linking and modulation of CD4 molecules through gp120 and anti-gp120. Experiments from Kion et al. (2) suggest that sequence homologies between gp160 and class II MHC molecules lead to immunodeficiency. In addition, a number of antigenic domains on gp160 are known to induce antibodies that enhance HIV-1 infection (3). Such effects known in context with gp160 could be avoided by using synthetic peptides or other subunit vaccines that only contain immunogenic and neutralizing epitopes as immunogens. Immunogenic peptides corresponding to parts of different viral proteins were already used for successful immunization (4,5,6). The use of synthetic peptides as immunogens offers a number of advantages. The antibodies produced have a predetermined specificity, and in the case of viruses, they can be selected to represent structures on the surface of virions. The synthetic polypeptides also are interesting in that they can induce antibody responses not seen under normal conditions. For example, it was found that in the hemagglutinin of influenza virus there are five major antigenic regions and that under conditions of natural infection the immune response includes antibodies only to these regions. With synthetic polypeptides, an immune response against other regions of the hemagglutinin polypeptide can be generated, and these antibodies have been found to be capable of neutralizing the virus. Therefore it is possible to induce neutralizing antibodies that have a broader reactivity than antibodies induced by whole proteins (4). In addition, immunizations with peptides derived from the nucleotide sequence of foot and mouth disease virus (FMDV) are described. In contrast to immunizations with the corresponding whole protein of FMDV, immunizations with these peptides lead to neutralizing antibodies which were also protective (5). Furthermore, a peptide containing part of the V3 loop of gp120 from the HIV-1 isolate HIV-1 IIIB was shown to induce a protective immune response against virus challenge with the same HIV-1 isolate (7,8).

Because synthetic peptides themselves have poor immunogenicity, they have to be coupled to molecules that provide an adjuvant effect such as tetanus toxoid or keyhole limpet hemocyan (5). Another possibility is to clone small peptides as fusion peptides with glutathione S-transferase of Schistosoma japonicum (9,10). In addition, attenuated viruses such as vaccinia, polio Sabin type 1 or influenza NA/B-NS can be used as vectors for immunogens. Vaccinia virus is used frequently as a vector of foreign genes of multiple pathogens. For example rabbits inoculated with recombinant vaccinia virus containing sequences from hepatitis B surface antigen (HBsAg), herpes simplex virus glycoprotein D, and influenza virus hemagglutinin produced antibodies to all three foreign antigens (11). Furthermore, a chimerical polio virus that expressed an epitope from gp41 of HIV-1 induced neutralizing antibodies against gp41 in rabbits (12). Since recently it is also possible to change the genome of influenza virus by *in vitro* mutagenesis (13). By means of this technique, it was possible to engineer a stable attenuated influenza A virus (14). In addition, by using this technique it was also possible to construct an intertypic chimerical virus, in which a six-amino-acid loop contained in the antigenic site B of the hemagglutinin of an H1 subtype was replaced by the corresponding structures of subtypes H2 and H3 (15). An advantage of influenza virus in this context is the availability of many variants so that repeated vaccination may be possible. Furthermore, influenza virus induces strong secretory and cellular immune responses, which may be advantageous for an anti-HIV-1 vaccine approach. In addition, it is unlikely that influenza virus is associated with the development of malignancies. There is no DNA phase involved in the replication of influenza viruses, which excludes the possibility of chromosomal integration of viral influenza genes.

The use of antiidiotypic antibodies is another possibility to achieve a specific immune reaction. Antiidiotypic antibodies are antibodies that specifically recognize and bind the antigen binding site of another antibody. As the combining sites of antibodies can be structurally looked at as a mirror image of the epitope that is bound, an antiidiotypic antibody

corresponds to the mirror image of this primary mirror image, which means that an antiidiotypic antibody displays the internal image of the epitope that is bound by the idiotypic antibody. Although one cannot always expect to find complete identity between the structure or the amino acid sequence respectively of the antiidiotypic antibody with that of the epitope, one can however see effects in practice that allow the conclusion that there is a structural, sequential or functional similarity between antiidiotypic antibodies and the respective epitopes. The use of antiidiotypic antibodies as a vaccine was initially proposed by Nisonoff and Lamoyi (16). In the case of African Sleeping Disease, it was first shown that a protective immune response against the causative agents, Trypanosoma brucei rhodesiense, could be elicited in BALB/c mice by vaccinating the mice with antiidiotypic antibody (17). In the case of viral antigens, the formation of antiidiotypic antibodies to a neutralizing epitope on the hemagglutinin molecule of Reovirus Type III was investigated. These antiidiotypic antibodies recognized the cellular receptor of Reovirus-hemagglutinin on both, cytolytic T-cells and neuronal cells, and were able to induce in mice a humoral as well as a cellular immune response specific to Reovirus-hemagglutinin (18, 19, 20).

DETAILED DESCRIPTION OF THE INVENTION

Peptides comprising 6 amino acid residues (aa) that bind specifically to the monoclonal antibody 2F5 were used as immunogens to induce neutralizing antibodies against HIV-1. For identification of these peptides overlapping fragments of gp41 (HIV-1 isolate BH10) were cloned as fusion peptides with glutathione transferase. The different fusion peptides were obtained through hybridization of gp41 corresponding oligonucleotides which were cloned between the Bam HI and the Eco RI site of the plasmid pGEX-2T (Pharmacia). The recombinant plasmids were transformed into the E. coli strain DH5α and expression of the fusion proteins was induced with isopropylthiogalactoside (IPTG). The E. coli extract was then purified with glutathione-sepharose 4B columns, loaded on sodiumdodecylsulfate-polyacrylamide gels, separated by electrophoresis and protein expression was analyzed by silver staining. Fusion peptides that were reactive with the monoclonal antibody 2F5 were identified by immunoblotting. Using this method peptides which bind to the monoclonal antibody 2F5 were identified: Figure 1 shows Western blots of fusion peptides with overlapping fragments of gp160 of HIV-1 (isolate BH10). In contrast to constructs that comprise aa 597 to 677, 634 to 677 and 648 to 677 (the numbering of amino acid residues corresponds to gp160 of HIV-1 isolate BH10, as described in the Swissprot database entry ENV$HIV10) which were reactive with the antibody 2F5, a fusion peptide comprising aa 667 to 677 did not show a positive reaction. This was the first indication that the epitope of the monoclonal antibody 2F5 is formed by aa within the sequence from position 648 to 667 of gp160. Based on these results, overlapping 6-mer peptides of this region were fused with the glutathione S-transferase. As shown in figure Ib the peptide containing the amino acid sequence GLU LEU ASP LYS TRP ALA (aa 662-667) was highly reactive with the antibody 2F5 whereas for peptides containing the amino acid sequence LEU ASP LYS TRP ALA SER (aa 663-668) or ASP LYS TRP ALA SER LEU (aa 664-669) reactivity with the monoclonal antibody was significantly lower. A peptide containing amino acid sequence LEU GLU LEU ASP LYS TRP (aa 661-666) showed no reactivity at all. These data suggest that the epitope of the monoclonal antibody comprises the amino acid sequence GLU LEU ASP LYS TRP ALA that correspond to aa 662-667 on gp160 of the HIV-1 BH10 isolate. In this context both, a synthetic peptide corresponding to this epitope sequence and a fusion protein containing this sequence were able to inhibit neutralization mediated by the 2F5 antibody (Fig. 4). Sequence comparison of that region revealed that the corresponding amino acid sequence is highly conserved between otherwise genetically highly divergent HIV-1 isolates (Table 2a). We also were able to show that fusion peptides with amino acid substitutions-according to different HIV-1 isolates- in this region were also reactive with the 2F5 antibody (Fig. 1c).

The presence of antigenic domains around this region has been reported previously (21,22). Teeuwsen et al. reported of a monoclonal antibody, that reacted with a peptide corresponding to aa 643 to 692 of gp160. In addition Broliden et al. reported that HIV-1 antibody-positive human sera were reactive with a peptide corresponding to region 657-671. However, in both cases a specific epitope was not identified. The monoclonal antibody reported by Teeuwsen et al. had no neutralizing activity. Also the sera reactive with the peptide 657-671 of Broliden et al. showed just partial neutralizing activity. In different neutralization assays this group was able to show neutralizing activity against HIV-1 isolate IIIB but not against SF2 and RF.

In contrast to this result, the monoclonal antibody 2F5 neutralizes a variety of different HIV-1 isolates including SF2 and RF (table 1). These data suggest that the antibodies of the sera reported by Broliden et al. as well as the monoclonal antibody reported by Teeuwsen et al. have a different specificity and recognize a different epitope than the antibody 2F5.

The application of the peptides described in the present invention as immunogen has several advantages. They comprise just 6 aa. Thus, other gp160 peptide sequences which induce antibodies that enhance HIV-1 infection or lead to immunosuppression can be avoided (2,3). Furthermore an effective HIV-1 vaccine should induce an immune response against HIV-1 isolates that vary considerably in their genomic sequences. In this context sequence comparison in the region of the 2F5 epitope revealed that the epitope of the 2F5 antibody is highly conserved between different

HIV-1 isolates (Table 2a). Since peptides with aa substitutions- corresponding to genetically different HIV-1 isolates- were reactive with the 2F5 antibody (Fig. 1c), it is likely that antibodies induced by peptides described in the present invention are directed against a variety of divergent HIV-1 isolates. In addition, the 2F5 antibody showed neutralizing activity against a wide variety of genetically different HIV-1 isolates which proves that peptides described in the present invention are presented as neutralizing epitopes (Table 1).

In order to know which variations of the epitope sequence are binding to the monoclonal antibody 2F5 we undertook a peptide mapping with a random hexapeptide library displayed on protein III of a filamentous phage (22a). The hexapeptide sequences of the eluted phage particles were compiled (Table 2b).

There is a wide range of variation in the progression of HIV-1 related disease in different HIV-1 infected persons. In many cases HIV-1 infection ends up in AIDS-related complex (ARC) and AIDS within some years, while some HIV-1 positive persons remain asymptomatic. It has been shown that antibody-titers against certain peptide epitopes are much lower in AIDS-patients compared to asymptomatic states (23). We found a significant correlation between the antibody-titers to the peptides described in the present invention and HIV-1 related disease progression (Fig. 2). Patients number 20,25,29,35,41,44 and 46 who have a high antibody-titer to peptides described in this invention (Fig. 2), did not show any progression in disease within the last five years so far. This means that generation of antibodies induced by peptides described in the present invention can inhibit or at least reduce the progression of HIV-1 related disease. The fact that there are rarely high antibody-titers to peptides described in this invention found in sera of HIV-1 positive patients indicates that these epitopes on gp160 are not recognized readily by the human immune system, resulting in low HIV-1 neutralizing antibody titers specific to these epitopes. An objective of the present invention is also to present the peptides described in the invention in a proper form and to induce a sufficient neutralizing immune response.

Example 1:

The cloning and expression of peptides described in the invention as fusion proteins with glutathione S-transferase (GST) and immunizations of mice with these peptides is described. All cloning methods were done according to standard procedures (24). Oligonucleotides corresponding to the peptides described in the invention were hybridized and cloned between the Bam HI and Eco RI site of the plasmid pGEX-2T (Pharmacia). By this the NH_2-terminal ends of these peptides were fused with the COOH-terminal ends of the GST. In addition, a stop codon was added to the COOH-terminal ends of the gp41 peptide sequences. These constructs were transformed into E. coli DH5α and expression of the fusion proteins was induced with isopropylthiogalactoside (IPTG). After three hours of induction bacteria were harvested by centrifugation, suspended in phosphate buffered saline (PBS, pH 7.2) containing 1 % Triton-X-100 and sonicated. Bacterial debris were spun down by centrifugation and the supernatant was loaded on glutathione-sepharose 4B columns (Pharmacia). Elution of the fusion proteins was done with 20mM glutathione and 120mM NaCl in 100mM Tris HCl (pH 8.0). Purified fusion proteins obtained with this procedure were used for immunizing mice according to standard procedures. As a control, mice were immunized with GST prepared in the same way as the fusion proteins. Sera from mice taken one week after the last immunization showed high neutralizing titers against peptides described in the invention and inhibited HIV-1 replication *in vitro* (Fig. 3b and 3c).

Example 2:

Example 2 describes the expression of peptide sequences described in the invention as part of the hemagglutinin of influenza A virus. *In vitro* mutagenesis was used to introduce this peptide sequence into the antigenic sites A,B,C, D and E of the hemagglutinin of influenza A virus (26, 27). These chimerical DNA-constructs were then "RNP-transfected" into influenza HK/WSN virus (13). These chimerical influenza/HIV viruses had the antigenic properties of said peptide. In antibody-adsorption experiments these chimerical viruses inhibited HIV-1 neutralization through the antibody 2F5 (Fig. 3a). Antisera of mice immunized with the chimerical viruses were reactive with said peptides (Fig. 3b). Furthermore, in vitro these antisera neutralized different HIV-1 isolates (Fig. 3c).

Example 3:

Example 3 describes the expression of peptides described in the invention as part of a so called "immunological supermolecule", in where the peptide sequence is inserted into the linker which connects the variable regions of the heavy and light chain of an immunoglobulin molecule. Specifically a single chain Fv construct of a neutralizing anti-HIV-gp120 antibody was made according to standard procedures (27) . In this construct peptide sequences described in the invention were inserted into the linker which connects the variable region of the light chain with the variable region of the heavy chain. This recombinant protein was expressed in E. coli and purified according to standard procedures. Two functions were observed with this construct. First this construct showed the antigen binding properties

of the original antibody and in addition this construct induced, when injected into mice, antibodies that neutralized different HIV-1 isolates (Fig. 3c).

This "immunological supermolecule" provides the possibility to obtain an active and passive immunization at the same time. Basically, in such a construct the antigen binding neutralizing properties of an antibody and the presentation of a neutralizing epitope are combined. In already HIV-1 infected persons the progression of infection could be slowed down with the first application by the antigen binding neutralizing properties, before the effective onset of the immune system is triggered by the neutralizing epitopes of this molecule. Thus the usual observed "time lag" between immunization and effective immune response of a typical active immunization could be overcome. In addition, it is most likely that during neutralization of already present HIV-1 virions the presentation of the epitope is very efficient.

Example 4:

Example 4 describes the formation of antiidiotypic antibodies to antibody 2F5 as well as the production of an antiidiotypic antibody by means of *in vitro* recombination techniques.

Antibody 2F5 was used to immunize mice in order to induce the formation of antiidiotypic antibody. The immunization scheme used was according to standard procedures in order to enhance the frequency at which antiidiotypic antibodies are developed in the animal. The polyclonal sera such obtained were tested for their immunoreactivity, whereby it was determined by means of antigen-competition ELISA that a part of the humoral immune response was indeed directed against the combining site of the antibody 2F5. Thus it was proven that in those sera antiidiotypic antibodies were present. In order to test the concept of vaccination by means of antiidiotypic antibodies, the sera containing antiidiotypic antibodies were subsequently used to immunize another group of mice. After completion of this immunization procedure, it was possible to detect an immune response to the antiidiotypic sera that was qualitatively comparable to the above described immune reaction against the HIV-1 peptide-part of the glutathione-S-transferase fusion protein described in example 1.

Since it was now proven, that the described peptide has the quality necessary to act as an immunogen, and since furthermore it had been shown that by using antiidiotypic antibodies with internal image quality of the described peptide it was possible to induce a HIV-1 neutralizing immune response, an antiidiotypic antibody was constructed by means of *in vitro* recombination techniques. In order to achieve this goal, one or more hypervariable regions (or parts thereof) of an existing, molecularly cloned antibody were substituted by peptide sequences described in the invention. The respective constructs where expressed as single chain Fv fragments in E. coli, and the recombinant proteins where purified according to standard methods. Immunization of mice with the antiidiotypic proteins such produced lead to a HIV-1 neutralizing immune response (Fig. 3c).

Example 5:

Example 5 describes the peptide mapping with a random hexapeptide library and immunizations of mice with phages containing peptides according to SEQ ID NO: 10 through SEQ ID NO: 25. The monoclonal antibody 2F5 was coated onto polystyrol tubes (Maxiscorp, Nunc, Denmark) at a concentration of 5µg/ml in coating buffer (0.1M Na-Carbonate buffer, pH 9.6) overnight at 4°C. After washing with PBS, the surface was blocked with PBS containing 5%w/v skimmed milk powder at 36°C for 2 hours. Washing with PBS was followed by incubation of a hexapeptide phage display library (10^{11} transduction units in TPBS (PBS including 0.5% v/v Tween 20) overnight at 5°C. Extensive washing with TPBS was followed by elution of phage with elution buffer (0.1N HCl/Glycine pH 2.2, 1mg BSA/ml). The eluate is neutralized with 1 M Tris and used for infection of E. coli K91Kan. Phage is prepared from the infected culture by the methods described (22a). The procedure is repeated 3 times. The final eluate was used to produce transduced E. coli K91Kan. DNA of these clones was sequenced and the respective phage displayed hexapeptide sequence was derived by computer translation.

Clones with the SEQ ID NO:10 through 25 (Table 2) were used for phage preparation and the respective phages were injected into mice. After two booster injections the respective sera were tested for HIV-1 neutralizing activity. All of these sera were neutralizing *in vitro*. The control serum was produced by immunization with wild-type phage f1 and did not neutralize HIV-1. In addition, oligonucleotides coding for SEQ ID NO: 10 through 25 were introduced into gene VIII of fd-tet between aa 27 and 28 of the immature protein VIII by standard cloning techniques. The recombinant phages were produced in E. coli K91Kan, purified by standard techniques (PEG mediated precipitation followed by CsCl gradient centrifugation) and used as immunogen. The respective sera were found to be neutralizing in HIV-1 neutralizing assays whereas anti-wild type fd-tet was not.

Sequence Listing:

SEQ ID NO: 1

LENGTH OF SEQUENCE: 6 amino acid residues
TYPE OF SEQUENCE: amino acid sequence
TYPE OF FRAGMENT: inner fragment

ORIGIN: GP160 of HIV-1 isolate BH10 POSITION OF THE SEQUENCE IN GP160: from residue 662 to 667

PROPERTIES: Epitope of a human monoclonal antibody directed against HIV-1 GP160

REFERENCE: translated from GenBank accession M15654, nucleotides 7563 to 7580

```
Glu Leu Asp Lys Trp Ala
1               5   6
```

SEQ ID NO: 2

LENGTH OF SEQUENCE: 6 amino acid residues
TYPE OF SEQUENCE: amino acid sequence
TYPE OF FRAGMENT: inner fragment

ORIGIN: GP160 of HIV-1 isolate JS4/26 POSITION OF THE SEQUENCE IN GP160: from residue 655 to 660

PROPERTIES: Epitope of a human monoclonal antibody directed against HIV-1 GP160

REFERENCE: translated from GenBank accession M37576, nucleotides 1963 to 1980

```
Glu Leu Asn Lys Trp Ala
1               5   6
```

SEQ ID NO: 3

LENGTH OF SEQUENCE: 6 amino acid residues
TYPE OF SEQUENCE: amino acid sequence
TYPE OF FRAGMENT: inner fragment

ORIGIN: GP160 of HIV-1 isolate (patient 3L) POSITION OF THE SEQUENCE IN GP41: from residue 164 to 169

PROPERTIES: Epitope of a human monoclonal antibody directed against HIV-1 GP160

REFERENCE: translated from GenBank accession X61352, nucleotides 490 to 507

```
Glu Leu Asp Lys Trp Asp
1               5   6
```

SEQ ID NO: 4

LENGTH OF SEQUENCE: 6 amino acid residues
TYPE OF SEQUENCE: amino acid sequence
TYPE OF FRAGMENT: inner fragment

ORIGIN: GP160 of HIV-1 isolate SF170 POSITION OF THE SEQUENCE IN GP160: from residue 667 to 672

PROPERTIES: Epitope of a human monoclonal antibody directed against HIV-1 GP160

REFERENCE: translated from GenBank accession M66533, nucleotides 1999 to 2016

Ala Leu Asp Lys Trp Ala
1 5 6

SEQ ID NO: 5

LENGTH OF SEQUENCE: 6 amino acid residues
TYPE OF SEQUENCE: amino acid sequence
TYPE OF FRAGMENT: inner fragment

ORIGIN: GP160 of HIV-1 isolate JH3 POSITION OF THE SEQUENCE IN GP160: from residue 673 to 678

PROPERTIES: Epitope of a human monoclonal antibody directed against HIV-1 GP160

REFERENCE: translated from GenBank accession M21138, nucleotides 2263 to 2280

Gly Leu Asp Lys Trp Ala
1 5 6

SEQ ID NO: 6

LENGTH OF SEQUENCE: 6 amino acid residues
TYPE OF SEQUENCE: amino acid sequence
TYPE OF FRAGMENT: inner fragment

ORIGIN: GP160 of HIV-1 isolate Z-84 POSITION OF THE SEQUENCE IN GP160: from residue 669 to 674

PROPERTIES: Epitope of a human monoclonal antibody directed against HIV-1 GP160

REFERENCE: translated from GenBank accession J03653, nucleotides 2037 to 2054

Gln Leu Asp Lys Trp Ala
1 5 6

SEQ ID NO: 7

LENGTH OF SEQUENCE: 6 amino acid residues
TYPE OF SEQUENCE: amino acid sequence
TYPE OF FRAGMENT: inner fragment

ORIGIN: GP160 of HIV-1 isolate CAM1 proviral genome POSITION OF THE SEQUENCE IN GP160: from residue 662 to 667

PROPERTIES: Epitope of a human monoclonal antibody directed against HIV-1 GP160

REFERENCE: translated from GenBank accession D10112, nucleotides 8209 to 8226

Glu Leu Asp Thr Trp Ala
1 5 6

SEQ ID NO: 8

LENGTH OF SEQUENCE: 6 amino acid residues
TYPE OF SEQUENCE: amino acid sequence
TYPE OF FRAGMENT: inner fragment

ORIGIN: GP160 of HIV-1 isolate JS4/6 POSITION OF THE SEQUENCE IN GP160: from residue 659 to 664

PROPERTIES: Epitope of a human monoclonal antibody directed against HIV-1 GP160

REFERENCE: translated from GenBank accession M37491, nucleotides 2416 to 2433

Ala Leu Asp Thr Trp Ala
1 5 6

SEQ ID NO: 9

LENGTH OF SEQUENCE: 6 amino acid residues
TYPE OF SEQUENCE: amino acid sequence
TYPE OF FRAGMENT: inner fragment

ORIGIN: GP160 of HIV-1 isolate SBB POSITION OF THE SEQUENCE IN GP160: from residue 413 to 418 (partial sequence)

PROPERTIES: Epitope of a human monoclonal antibody directed against HIV-1 GP160

REFERENCE: translated from GenBank accession M77229, nucleotides 1239 to 1256

Lys Leu Asp Glu Trp Ala
1 5 6

SEQ ID NO: 10

LENGTH OF SEQUENCE: 6 amino acid residues
TYPE OF SEQUENCE: amino acid sequence
TYPE OF FRAGMENT: inner fragment

ORIGIN: p3 fusion protein of filamentous phage fUSE5 POSITION OF THE SEQUENCE IN p3: from residue 4 to 9

PROPERTIES: hexapeptide binding to monoclonal antibody 2F5

Ser Leu Asp Lys Trp Ala
1 5 6

SEQ ID NO: 11

LENGTH OF SEQUENCE: 6 amino acid residues
TYPE OF SEQUENCE: amino acid sequence
TYPE OF FRAGMENT: inner fragment

ORIGIN: p3 fusion protein of filamentous phage fUSE5 POSITION OF THE SEQUENCE IN p3: from residue 4 to 9

PROPERTIES: hexapeptide binding to monoclonal antibody 2F5

```
Gly Arg Asp Lys Trp Ala
1               5   6
```

SEQ ID NO: 12

LENGTH OF SEQUENCE: 6 amino acid residues
TYPE OF SEQUENCE: amino acid sequence
TYPE OF FRAGMENT: inner fragment

ORIGIN: p3 fusion protein of filamentous phage fUSE5 POSITION OF THE SEQUENCE IN p3: from residue 4 to 9

PROPERTIES: hexapeptide binding to monoclonal antibody 2F5

```
Gly Ala Asp Lys Trp Ala
1               5   6
```

SEQ ID NO: 13

LENGTH OF SEQUENCE: 6 amino acid residues
TYPE OF SEQUENCE: amino acid sequence
TYPE OF FRAGMENT: inner fragment

ORIGIN: p3 fusion protein of filamentous phage fUSE5 POSITION OF THE SEQUENCE IN p3: from residue 4 to 9

PROPERTIES: hexapeptide binding to monoclonal antibody 2F5

```
Ala His Glu Lys Trp Ala
1               5   6
```

SEQ ID NO: 14

LENGTH OF SEQUENCE: 6 amino acid residues
TYPE OF SEQUENCE: amino acid sequence
TYPE OF FRAGMENT: inner fragment

ORIGIN: p3 fusion protein of filamentous phage fUSE5 POSITION OF THE SEQUENCE IN p3: from residue 4 to 9

PROPERTIES: hexapeptide binding to monoclonal antibody 2F5


```
Ala Cys Asp Gln Trp Ala
1               5   6
```

SEQ ID NO: 15

LENGTH OF SEQUENCE: 6 amino acid residues
TYPE OF SEQUENCE: amino acid sequence
TYPE OF FRAGMENT: inner fragment

ORIGIN: p3 fusion protein of filamentous phage fUSE5 POSITION OF THE SEQUENCE IN p3: from residue 4 to 9

PROPERTIES: hexapeptide binding to monoclonal antibody 2F5

```
Gly Ala Asp Lys Trp Gly
1               5   6
```

SEQ ID NO: 16

LENGTH OF SEQUENCE: 6 amino acid residues
TYPE OF SEQUENCE: amino acid sequence
TYPE OF FRAGMENT: inner fragment

ORIGIN: p3 fusion protein of filamentous phage fUSE5 POSITION OF THE SEQUENCE IN p3: from residue 4 to 9

PROPERTIES: hexapeptide binding to monoclonal antibody 2F5

```
Gly Ala Asp Lys Trp Asn
1               5   6
```

SEQ ID NO: 17

LENGTH OF SEQUENCE: 6 amino acid residues
TYPE OF SEQUENCE: amino acid sequence
TYPE OF FRAGMENT: inner fragment

ORIGIN: p3 fusion protein of filamentous phage fUSE5 POSITION OF THE SEQUENCE IN p3: from residue 4 to 9

PROPERTIES: hexapeptide binding to monoclonal antibody 2F5

```
Gly Ala Asp Lys Trp Cys
1               5   6
```

SEQ ID NO: 18

LENGTH OF SEQUENCE: 6 amino acid residues
TYPE OF SEQUENCE: amino acid sequence

TYPE OF FRAGMENT: Inner fragment

ORIGIN: p3 fusion protein of filamentous phage fUSE5 POSITION OF THE SEQUENCE IN p3: from residue 4 to 9

PROPERTIES: hexapeptide binding to monoclonal antibody 2F5

```
Gly Ala Asp Lys Trp Val
1               5   6
```

SEQ ID NO: 19

LENGTH OF SEQUENCE: 6 amino acid residues
TYPE OF SEQUENCE: amino acid sequence
TYPE OF FRAGMENT: inner fragment

ORIGIN: p3 fusion protein of filamentous phage fUSE5 POSITION OF THE SEQUENCE IN p3: from residue 4 to 9

PROPERTIES: hexapeptide binding to monoclonal antibody 2F5

```
Gly Ala Asp Lys Trp His
1               5   6
```

SEQ ID NO: 20

LENGTH OF SEQUENCE: 6 amino acid residues
TYPE OF SEQUENCE: amino acid sequence
TYPE OF FRAGMENT: inner fragment

ORIGIN: p3 fusion protein of filamentous phage fUSE5 POSITION OF THE SEQUENCE IN p3: from residue 4 to 9

PROPERTIES: hexapeptide binding to monoclonal antibody 2F5

```
Gly Ala Asp Lys Cys His
1               5   6
```

SEQ ID NO: 21

LENGTH OF SEQUENCE: 6 amino acid residues
TYPE OF SEQUENCE: amino acid sequence
TYPE OF FRAGMENT: inner fragment

ORIGIN: p3 fusion protein of filamentous phage fUSE5 POSITION OF THE SEQUENCE IN p3: from residue 4 to 9

PROPERTIES: hexapeptide binding to monoclonal antibody 2F5


```
Gly Ala Asp Lys Cys Gln
1               5   6
```

SEQ ID NO: 22

LENGTH OF SEQUENCE: 6 amino acid residues
TYPE OF SEQUENCE: amino acid sequence
TYPE OF FRAGMENT: inner fragment

ORIGIN: p3 fusion protein of filamentous phage fUSE5 POSITION OF THE SEQUENCE IN p3: from residue 4 to 9

PROPERTIES: hexapeptide binding to monoclonal antibody 2F5

```
Ala Tyr Asp Lys Trp Ser
1               5   6
```

SEQ ID NO: 23

LENGTH OF SEQUENCE: 6 amino acid residues
TYPE OF SEQUENCE: amino acid sequence
TYPE OF FRAGMENT: inner fragment

ORIGIN: p3 fusion protein of filamentous phage fUSE5 POSITION OF THE SEQUENCE IN p3: from residue 4 to 9

PROPERTIES: hexapeptide binding to monoclonal antibody 2F5

```
Ala Phe Asp Lys Trp Val
1               5   6
```

SEQ ID NO: 24

LENGTH OF SEQUENCE: 6 amino acid residues
TYPE OF SEQUENCE: amino acid sequence
TYPE OF FRAGMENT: inner fragment

ORIGIN: p3 fusion protein of filamentous phage fUSE5 POSITION OF THE SEQUENCE IN p3: from residue 4 to 9

PROPERTIES: hexapeptide binding to monoclonal antibody 2F5

```
Gly Pro Asp Lys Trp Gly
1               5   6
```

SEQ ID NO: 25

LENGTH OF SEQUENCE: 6 amino acid residues

TYPE OF SEQUENCE: amino acid sequence
TYPE OF FRAGMENT: inner fragment

ORIGIN: p3 fusion protein of filamentous phage fUSE5 POSITION OF THE SEQUENCE IN p3: from residue 4 to 9

PROPERTIES: hexapeptide binding to monoclonal antibody 2F5

Ala Arg Asp Lys Trp Ala
1 5 6

SEQ ID NO: 26

LENGTH of SEQUENCE: 18 base pairs
TYPE OF SEQUENCE: nucleotide sequence

TYPE OF STRAND: single strand
TOPOLOGY OF SEQUENCE: linear
TYPE OF SEQUENCED MOLECULE: cDNA to viral RNA
TYPE OF FRAGMENT: inner fragment
ORIGIN: GP160 of HIV-1 isolate BH10
REFERENCE: GenBank accession M15654
POSITION OF THE SEQUENCE IN DATABASE ENTRY: 7564-7580

gaattagata aatgggca
18
1 11

SEQ ID NO: 27

LENGTH of SEQUENCE: 18 base pairs
TYPE OF SEQUENCE: nucleotide sequence

TYPE OF STRAND: single strand
TOPOLOGY OF SEQUENCE: linear
TYPE OF SEQUENCED MOLECULE: cDNA to viral RNA
TYPE OF FRAGMENT: inner fragment
ORIGIN:GP160 of HIV-1 isolate JS4/26
REFERENCE: GenBank accession M37576
POSITION OF THE SEQUENCE IN DATABASE ENTRY: 1963-1980

gaattgaata agtgggca
18
1 11

SEQ ID NO: 28

LENGTH of SEQUENCE: 18 base pairs
TYPE OF SEQUENCE: nucleotide sequence

TYPE OF STRAND: single strand
TOPOLOGY OF SEQUENCE: linear
TYPE OF SEQUENCED MOLECULE: cDNA to viral RNA
TYPE OF FRAGMENT: inner fragment
ORIGIN: GP160 of HIV-1 isolate (patient 3L)
REFERENCE: GenBank accession X61352
POSITION OF THE SEQUENCE IN DATABASE ENTRY: 490-507

gaattagata agtgggac
18
1 11

SEQ ID NO: 29

LENGTH of SEQUENCE: 18 base pairs
TYPE OF SEQUENCE: nucleotide sequence

TYPE OF STRAND: single strand
TOPOLOGY OF SEQUENCE: linear
TYPE OF SEQUENCED MOLECULE: cDNA to viral RNA
TYPE OF FRAGMENT: inner fragment
ORIGIN: GP160 of HIV-1 isolate SF170
REFERENCE: GenBank accession M66533
POSITION OF THE SEQUENCE IN DATABASE ENTRY: 1999-2016

gcattggaca agtgggca
18
1 11

SEQ ID NO: 30

LENGTH of SEQUENCE: 18 base pairs
TYPE OF SEQUENCE: nucleotide sequence

TYPE OF STRAND: single strand
TOPOLOGY OF SEQUENCE: linear
TYPE OF SEQUENCED MOLECULE: cDNA to viral RNA
TYPE OF FRAGMENT: inner fragment
ORIGIN: GP160 of HIV-1 isolate JH3
REFERENCE: GenBank accession M21138
POSITION OF THE SEQUENCE IN DATABASE ENTRY: 2263-2280

gggttagata aatgggca
18
1 11

SEQ ID NO: 31

LENGTH of SEQUENCE: 18 base pairs
TYPE OF SEQUENCE: nucleotide sequence

TYPE OF STRAND: single strand
TOPOLOGY OF SEQUENCE: linear
TYPE OF SEQUENCED MOLECULE: cDNA to viral RNA
TYPE OF FRAGMENT: inner fragment
ORIGIN: GP160 of HIV-1 isolate Z-84
REFERENCE: GenBank accession J03653
POSITION OF THE SEQUENCE IN DATABASE ENTRY: 2037-2054

caattggaca aatgggca
18
1 11

SEQ ID NO: 32

LENGTH of SEQUENCE: 18 base pairs
TYPE OF SEQUENCE: nucleotide sequence

TYPE OF STRAND: single strand
TOPOLOGY OF SEQUENCE: linear
TYPE OF SEQUENCED MOLECULE: cDNA to viral RNA
TYPE OF FRAGMENT: inner fragment
ORIGIN: GP160 of HIV-1 isolate CAM1 proviral genome
REFERENCE: GenBank accession D10112
POSITION OF THE SEQUENCE IN DATABASE ENTRY: 8209-8226

gaattggata cgtgggca
18
1 11

SEQ ID NO: 33

LENGTH of SEQUENCE: 18 base pairs
TYPE OF SEQUENCE: nucleotide sequence

TYPE OF STRAND: single strand
TOPOLOGY OF SEQUENCE: linear
TYPE OF SEQUENCED MOLECULE: cDNA to viral RNA
TYPE OF FRAGMENT: inner fragment
ORIGIN: GP160 of HIV-1 isolate JS4/6
REFERENCE: GenBank accession M37491
POSITION OF THE SEQUENCE IN DATABASE ENTRY: 2416-2433

gcattggata cgtgggca
18
1 11

SEQ ID NO: 34

LENGTH of SEQUENCE: 18 base pairs
TYPE OF SEQUENCE: nucleotide sequence

TYPE OF STRAND: single strand
TOPOLOGY OF SEQUENCE: linear
TYPE OF SEQUENCED MOLECULE: cDNA to viral RNA
TYPE OF FRAGMENT: inner fragment
ORIGIN: GP160 of HIV-1 isolate SBB
REFERENCE: GenBank accession M77229
POSITION OF THE SEQUENCE IN DATABASE ENTRY: 1239-1256

aagttagatg agtgggca
18
1 11

SEQ ID NO: 35

LENGTH of SEQUENCE: 18 base pairs
TYPE OF SEQUENCE: nucleotide sequence

TYPE OF STRAND: single strand
TOPOLOGY OF SEQUENCE: linear
TYPE OF SEQUENCED MOLECULE: phage DNA
TYPE OF FRAGMENT: inner fragment
ORIGIN: gene coding for p3 fusion protein of filamentous phage fUSE5

POSITION OF THE SEQUENCE IN p3: 10 to 27

tcgcttgata agtgggcc
18
1 11

SEQ ID NO: 36

LENGTH of SEQUENCE: 18 base pairs
TYPE OF SEQUENCE: nucleotide sequence

TYPE OF STRAND: single strand
TOPOLOGY OF SEQUENCE: linear
TYPE OF SEQUENCED MOLECULE: phage DNA
TYPE OF FRAGMENT: inner fragment
ORIGIN: gene coding for p3 fusion protein of filamentous phage fUSE5

POSITION OF THE SEQUENCE IN p3: 10 to 27

gggcgtgata agtgggcg
18
1 11

SEQ ID NO: 37

LENGTH of SEQUENCE: 18 base pairs
TYPE OF SEQUENCE: nucleotide sequence

TYPE OF STRAND: single strand
TOPOLOGY OF SEQUENCE: linear
TYPE OF SEQUENCED MOLECULE: phage DNA
TYPE OF FRAGMENT: inner fragment
ORIGIN: gene coding for p3 fusion protein of filamentous phage fUSE5

POSITION OF THE SEQUENCE IN p3: 10 to 27

ggggctgata agtgggcg
18
1 11

SEQ ID NO: 38

LENGTH of SEQUENCE: 18 base pairs
TYPE OF SEQUENCE: nucleotide sequence

TYPE OF STRAND: single strand
TOPOLOGY OF SEQUENCE: linear
TYPE OF SEQUENCED MOLECULE: phage DNA
TYPE OF FRAGMENT: inner fragment
ORIGIN: gene coding for p3 fusion protein of filamentous phage fUSE5

POSITION OF THE SEQUENCE IN p3: 10 to 27

gctcatgaaa agtgggcg
18
1 11

SEQ ID NO: 39

LENGTH of SEQUENCE: 18 base pairs
TYPE OF SEQUENCE: nucleotide sequence

TYPE OF STRAND: single strand
TOPOLOGY OF SEQUENCE: linear
TYPE OF SEQUENCED MOLECULE: phage DNA
TYPE OF FRAGMENT: inner fragment
ORIGIN: gene coding for p3 fusion protein of filamentous phage fUSE5

POSITION OF THE SEQUENCE IN p3: 10 to 27

gcttgtgatc agtgggcg
18
1 11

SEQ ID NO: 40

LENGTH of SEQUENCE: 18 base pairs
TYPE OF SEQUENCE: nucleotide sequence

TYPE OF STRAND: single strand
TOPOLOGY OF SEQUENCE: linear
TYPE OF SEQUENCED MOLECULE: phage DNA
TYPE OF FRAGMENT: inner fragment
ORIGIN: gene coding for p3 fusion protein of filamentous phage fUSE5

POSITION OF THE SEQUENCE IN p3: 10 to 27

ggagctgata agtggggt
18
1 11

SEQ ID NO: 41

LENGTH of SEQUENCE: 18 base pairs
TYPE OF SEQUENCE: nucleotide sequence

TYPE OF STRAND: single strand
TOPOLOGY OF SEQUENCE: linear
TYPE OF SEQUENCED MOLECULE: phage DNA
TYPE OF FRAGMENT: inner fragment
ORIGIN: gene coding for p3 fusion protein of filamentous phage fUSE5

POSITION OF THE SEQUENCE IN p3: 10 to 27

ggagctgata agtggaat
18
1 11

SEQ ID NO: 42

LENGTH of SEQUENCE: 18 base pairs
TYPE OF SEQUENCE: nucleotide sequence

TYPE OF STRAND: single strand
TOPOLOGY OF SEQUENCE: linear
TYPE OF SEQUENCED MOLECULE: phage DNA
TYPE OF FRAGMENT: inner fragment
ORIGIN: gene coding for p3 fusion protein of filamentous phage fUSE5

POSITION OF THE SEQUENCE IN p3: 10 to 27

ggcgctgata aatggtgt
18
1 11

SEQ ID NO: 43

LENGTH of SEQUENCE: 18 base pairs
TYPE OF SEQUENCE: nucleotide sequence

TYPE OF STRAND: single strand
TOPOLOGY OF SEQUENCE: linear
TYPE OF SEQUENCED MOLECULE: phage DNA
TYPE OF FRAGMENT: inner fragment
ORIGIN: gene coding for p3 fusion protein of filamentous phage fUSE5

POSITION OF THE SEQUENCE IN p3: 10 to 27

ggcgctgata aatgggtt
18
1 11

SEQ ID NO: 44

LENGTH of SEQUENCE: 18 base pairs
TYPE OF SEQUENCE: nucleotide sequence

TYPE OF STRAND: single strand

TOPOLOGY OF SEQUENCE: linear

TYPE OF SEQUENCED MOLECULE: phage DNA

TYPE OF FRAGMENT: inner fragment

ORIGIN: gene coding for p3 fusion protein of filamentous phage fUSE5

POSITION OF THE SEQUENCE IN p3: 10 to 27

ggggctgata agtggcat
18
1 11

SEQ ID NO: 45

LENGTH of SEQUENCE: 18 base pairs
TYPE OF SEQUENCE: nucleotide sequence

TYPE OF STRAND: single strand

TOPOLOGY OF SEQUENCE: linear
TYPE OF SEQUENCED MOLECULE: phage DNA
TYPE OF FRAGMENT: inner fragment
ORIGIN: gene coding for p3 fusion protein of filamentous phage fUSE5

POSITION OF THE SEQUENCE IN p3: 10 to 27

ggagctgata aatgtcat
18
1 11

SEQ ID NO: 46

LENGTH of SEQUENCE: 18 base pairs
TYPE OF SEQUENCE: nucleotide sequence

TYPE OF STRAND: single strand
TOPOLOGY OF SEQUENCE: linear
TYPE OF SEQUENCED MOLECULE: phage DNA
TYPE OF FRAGMENT: inner fragment
ORIGIN: gene coding for p3 fusion protein of filamentous phage fUSE5

POSITION OF THE SEQUENCE IN p3: 10 to 27

ggagctgata aatgtcag
18
1 11

SEQ ID NO: 47

LENGTH of SEQUENCE: 18 base pairs
TYPE OF SEQUENCE: nucleotide sequence

TYPE OF STRAND: single strand
TOPOLOGY OF SEQUENCE: linear
TYPE OF SEQUENCED MOLECULE: phage DNA
TYPE OF FRAGMENT: inner fragment
ORIGIN: gene coding for p3 fusion protein of filamentous phage fUSE5

POSITION OF THE SEQUENCE IN p3: 10 to 27

gcttatgata agtggagt
18
1 11

SEQ ID NO: 48

LENGTH of SEQUENCE: 18 base pairs
TYPE OF SEQUENCE: nucleotide sequence

TYPE OF STRAND: single strand
TOPOLOGY OF SEQUENCE: linear
TYPE OF SEQUENCED MOLECULE: phage DNA
TYPE OF FRAGMENT: inner fragment
ORIGIN: gene coding for p3 fusion protein of filamentous phage fUSE5

POSITION OF THE SEQUENCE IN p3: 10 to 27

gcttttgata agtgggtt
18
1 11

SEQ ID NO: 49

LENGTH of SEQUENCE: 18 base pairs
TYPE OF SEQUENCE: nucleotide sequence

TYPE OF STRAND: single strand
TOPOLOGY OF SEQUENCE: linear
TYPE OF SEQUENCED MOLECULE: phage DNA
TYPE OF FRAGMENT: inner fragment
ORIGIN: gene coding for p3 fusion protein of filamentous phage fUSE5

POSITION OF THE SEQUENCE IN p3: 10 to 27

gggcctgata aatggggt
18
1 11

SEQ ID NO: 50

LENGTH of SEQUENCE: 18 base pairs
TYPE OF SEQUENCE: nucleotide sequence

TYPE OF STRAND: single strand
TOPOLOGY OF SEQUENCE: linear
TYPE OF SEQUENCED MOLECULE: phage DNA
TYPE OF FRAGMENT: inner fragment
ORIGIN: gene coding for p3 fusion protein of filamentous phage fUSE5

POSITION OF THE SEQUENCE IN p3: 10 to 27

gctcgtgata agtgggcg
18
1 11

Literature

1. Mittler, R.S., and K.Hoffmann. 1989. Synergism between HIV GP120 and gp 120-specific antibody in blocking

human T cell activation, Science 245: 1380-1382

2. Kion, T.A., and G.W. Hoffmann. 1991. Anti-HIV and anti-anti-MHC antibodies in alloimmune and autoimmune mice, Science 253: 1138-1140

3. Jiang S., K. Lin and A.R. Neurath. 1991. Enhancement of human immunodefeciency virus type 1 infection by antisera to peptides from the envelope glycoproteins gp120/gp41, J.Exp.Med. USA 174: 1557-1563

4. Green, N., H.Alexander, A. Olson, S.Alexander, T. M. Shinnck, J.G. Sutcliffe, and R.A. Lerner. 1982. Immunogenic Structure of the Influenza Virus Hemagglutinin, Cell 28: 477-487

5. Bittle, J.L., R.A. Houghten, H. Alexander, T.M. Shinnick, J.G.Sutcliffe and R.A. Lerner. 1982. Protection against Foot-and-mouth disease by immunization with a chemically synthesized peptide predicted from the viral nucleotide sequence, Nature 298: 30-33

6. R. A.Lerner. 1982. Tapping the immunological repertoire to produce antibodies of predetermined specificity, Nature 299: 592-596

7. Wahren, B., G. Bratt, J. Hinkula, G. Gilljam, S. Nordlund, P.A. Broliden, L. Akerblom, B. Morein, and E. Sandström.1990. Monoclonal antibodies given as passive treatment to HIV-infected individuals, Cinquieme Colloque des Cent Gardes :263-268

8. Emini, E.A., W.A. Schleif, J.H. Nunberg, A.J. Conley, Y. Eda, S. Tokiyoshi, S.D. Putney, S. Matsushita, K.E. Cobb, C.M. Jett, J.W. Eichberg, and K.K. Murthy. 1992. Prevention of HIV-1 infection in chimpanzees by gp 120 V3 domain- specific monoclonal antibody, Nature 355: 728-730

9. Fikrig E., S.W. Barthold, F.S. Kantor,, and R.A. Flavell. 1990. Protection of mice against the lyme disease agent by immunizing with recombinant OspA, Science 250: 553-556

10. Johnson K.S., G.B.L. Harrison, M.W. Lightowlers, K.L. O'Hoy, W.G. Cougle, R.P. Dempster, S.B. Lawrence, J.G. Vinton, D.D. Heath, and M.D. Rickard. 1989. Vaccination against ovine cysticercosis using a defined recombinant antigen, Nature 338: 585-587.

11. Perkus, M.E., A. Piccini, R.R. Lipinskas, and E. Paoletti. 1985. Recombinant vaccinia virus:Immunization against multiple pathogens, Science 229: 981-984

12. Evans J.D., J.McKeating, J.M. Meredith, K.L. Burke, K. Katrak,l A. John, M. Ferguson, P.D.Minor, R.A. Weiss, and J.W. Almond. 1989. An engineered Poliovirus chimaera elicits broadly reactive HIV-1 neutralizing antibodies, Nature 339: 385-388.

13. Enami M., W.Luytjes, M. Krystal, and P.Palese et al. 1990. Introduction of site-specific mutations into the genome of Influenza virus, Proc. Natl. Acad. Sci. USA 87: 3802-3805.

14. Muster T., E.K.Subbarao, M.Enami, B.R. Murphy, and P. Palese. 1991. An Influenza A virus containing Influenza B virus 5' and 3' noncoding regions on the neuraminidase gene is attenuated in mice, Proc.Natl. Acad. Sci. USA 88: 5177-5181.

15. Li, S, J.L. Schulmann, T. Moran, C.Bona, and P.Palese. 1992. Influenza-A virus transfectants with chimeric Hemagglutinins containing epitopes from different subtypes, J.Virol.66.: 399-404

16. Nisonoff A., E. Lamoyi. 1981. Clin. Immunol. Immunopathol. 21: 397

17. Sacks D.L., K.M. Esser and A. Sher. 1982. J. Exp. Med. 155: 1108

18. Kauffman R.S., J.H. Noseworthy, J.T. Nepom, R. Finberg, B.N. Fields, and M.I. Greene. 1983. J. Immunol. 131: 2539

19. Sharpe A.H., G.N. Gaulton, K.K. McDade, B.N. Fields, and M.I. Greene. 1984. J. Exp. Med. 160: 1195

20. Gaulton G.N., and M.I. Greene. 1986. Ann. Rev. Immunol. 4: 253

21. Broliden, P., A. Gegerfelt, P. Clapham, J. Rosen, E. Fenyö, B. Wahren, and K. Broliden. 1992. Identification of human neutralizing regions of the human immunodeficiency virus type-1 envelope glycoproteins, Proc. Natl. Acad. Sci. USA 89/2:461-465.

22. Teeuwsen V.J., K.H. Siebelnik, S. Crush-Stanton, A.D.B. Swerdlow, J.J. Schalken, J. Goudsmit, R. Van De Akker, M.J. Stukart, F.G. Uytdehaag, and A.D. Osterhaus. 1990. Production and characterization of a human monoclonal antibody, reactive with conserved epitope on gp41 of human immunodeficiency virus type 1, AIDS Res Hum Retroviruses 6: 381-392.

22a. Scott, J. K. and G. P. Smith. 1990. Searching for peptide ligands with an epitope library, Science 249: 386-390

23. Neurath, A.R., N. Strick, P.Taylor, P. Rubinstein, and C.E. Stevens. 1990. Search for epitope- specific antibody responses to the human immunodeficiency virus (HIV-1) envelope glycoproteins signifying resistance to desease development, AIDS Res Hum Retroviruses 6: 1183-1191

24. Sambrook, J., E. F. Fritsch and T. Maniatis. 1989. Molecular Cloning. A Laboratory manual. Cold Spring Harbor Laboratory Press, New York

25. Wharton, S.A., W. Weis, J.J. Shekel, and D.C. Wiley. 1989. Strucutre, function and antigenicity of the Hemagglutinin of Influenza virus, in: R.M. Krug (ed), The Influenza Viruses, Plenum Press, New York

26. Wiley, D.C., I.A. Wilson and J.J. Shekel. 1981. Structural identification of the antibody binding sites of Hong Kong Influenza Hemagglutinin and their involvement in the antigenic variation, Nature 289: 373-378

27. Kohl, J., F. Rüker, G. Himmler, E. Razazzi, and H. Katinger. 1991. Cloning and expression of a HIV-1 specific single-chain Fv region fused to Escherichia coli Alkaline Phosphatase, Ann. New York Acad. Sci. 646: 106-114

Tables

Table 1:

Neutralizing properties of human monoclonal antibody 2F5						
a) *in vitro* neutralization assays:						
	Isolate					
	IIIB	MN	RF	SF2	A	C
number of positive tests	8/8	2/2	2/2	n.t.	8/8	4/4
neutralizing concentration (µg/ml)	10	10	10		50	10
b)syncitia inhibition assay:						
	Isolate					
	IIIB	MN	RF	SF2	A	C
number of positive tests	18/18	11/11	6/10	1/1	1/1	2/3
EC_{50} (µg/ml)	12,8	12	13,7	1,9	27	10

a) *in vitro* neutralization assay: Different concentrations of the 2F5 antibody were incubated with cellfree virus preparations (10^2-10^3 $TCID_{50}$) 1 h at 37°C. Aliquots of 10^5 H9 cells were added to virus/antibody mixtures and incubated for an additional hour at 37°C. After 20 days p24 antigen concentration as indicator for virus replication was determined from supernatants according to standard procedures.

b) Syncytia inhibition assay: Antibody/virus mixtures were prepared as described in table Ia. To this mixtures 10^5 AA2 cells were added and incubated at 37°C. After 5 days syncitia formation as indicator for HIV-1 replication was evaluated. Abbrevations:A and C are clinical isolates from Vienna

a)	|E|L|D|K|W|A|	43
	|A|:|:|:|:|:|	5
	|:|:|N|:|:|:|	1
	|:|:|:|:|:|D|	2
	|A|:|:|T|:|:|	3
	|Q|:|:|:|:|:|	2
	|:|:|:|T|:|:|	1
	|G|:|:|:|:|:|	1
	|K|:|:|E|:|:|	1
	|-|-|-|-|-|-|---	
b)	|S|:|:|:|:|:|	
	|G|R|:|:|:|:|	
	|G|A|:|:|:|:|	
	|A|H|E|:|:|:|	
	|A|C|:|Q|:|:|	
	|G|A|:|:|:|G|	
	|G|A|:|:|:|N|	
	|G|A|:|:|:|C|	
	|G|A|:|:|:|V|	
	|G|A|:|:|:|H|	
	|G|A|:|:|C|H|	
	|G|A|:|:|C|Q|	
	|A|Y|:|:|:|S|	
	|A|F|:|:|:|V|	
	|G|P|:|:|:|G|	
	|A|R|:|:|:|A|	

conserved between different isolates

random hexapeptide phage display

2F5 - binding.

Table 2:Peptide sequences bound by antibody 2F5:

a: sequences present on gp160 of different HIV-1 isolates. The number on the right side of each sequence indicates the number of incidences in the databases that were screened (SwissProt and GenPept).

b: binding sequences found by screening a random hexapeptide library expressed on the surface of filamentous phage (sequences already described in a) are not included).

Figure legends:

Fig.1: Western blots of fusion peptides. Recombinant proteins expressed in E. coli were purified as described in example 1 and 100ng of each fusion peptide was fractionated by sodium dodecyl sulfate polyacrylamide gel electrophoresis on a 20% polyacrylamidegel and electroblotted onto a nitrocellulose filter. The blots were blocked with 0.5% nonfat dried milk in phosphate-buffered saline containing 0.1% Tween for 1h at room temperature. After washing, blots were incubated with antibody 2F5 (500ng/ml) for 1h at room temperature. After washing, blots were incubated for 1 h at room temperature with anti-human IgG-alkaline-phosphatase-conjugat. Blots were developed with 1M diethanolaminbuffer(pH9.6) containing 350ug/ml nitro-blue tetrazolium chloride and 350 ug/ml 5-bromo-4-chloro-3-indolyl-phosphate.
Fig.1a: Lane 1, glutathion S-transferase (GST); lane 2, amino acids (aa) 597-677 of gp160 fused with GST; lane 3, aa 634-677 fused with GST; lane 4, aa 648-677 fused with GST; lane 5 aa 667-677 fused with GST.
Fig.1b: lane 1, GST; lane 2, GST fused with aa GLU LEU ASP LYS TRP ALA (aa 662-667); lane 3, GST with aa LEU ASP LYS TRP ALA SER (aa 663-668); lane 4, GST with ASP LYS TRP ALA SER LEU (aa 664-669); lane 5, GST with aa LEU GLU LEU ASP LYS TRP (aa 661-666)
Fig.1c: Fusionpeptides with amino acid substitutions according to HIV-1 isolates with differences in the region of the 2F5 epitope. Amino acid differences are underlined. Lane 1, GST; lane 2, GLU LEU ASP LYS TRP ALA; lane 3, GLN LEU ASP LYS TRP ALA; lane 4, GLY LEU ASP LYS TRP ALA; lane 5, ALA LEU ASP LYS TRP ALA; lane 6, GLU LEU ASN LYS TRP ALA (reaction of this fusion peptide with the 2F5 antibody is not visible in this Western blot; however in ELISA competition assays this peptide was competitive to recombinant gp41 for binding to the 2F5 antibody); lane 7, GLU LEU ASP THR TRP ALA; lane 8, GLU LEU ASP LYS TRP ASP

Claims

Claims for the following Contracting States : BE, CH, DE, DK, FR, GB, IE, IT, LI, NL, PT, SE

1. Peptides binding to antibodies that show neutralizing activity against different strains and clinical isolates of HIV-1 and that inhibit the fusion of cells caused by HIV-1, characterised in that said peptides are composed according to anyone of the SEQ ID NO: 1 through SEQ ID NO: 25.

2. Peptides according to claim 1, characterised in that they are genetically encoded by the nucleotide sequence according to anyone of the SEQ ID NO: 26 through SEQ ID NO: 50 or by sequences that are deduced from SEQ ID NO: 26 through SEQ ID NO: 50 by degeneration.

3. Peptides according to claim 1 or 2, which upon injection into a mammal either alone or in combination with an adjuvant cause an immune response that leads to the generation of HIV-1 neutralizing antibodies.

4. Peptides according to anyone of claims 1 to 3 in combination with an adjuvant, wherein the adjuvant is a substance to which said peptides are bound through chemical interaction.

5. Peptides according to claim 4 in the form of fusion peptides, characterised in that a protein or part of a protein is used as the adjuvant, to which said peptides are bound by fusion of the respective nucleotide sequences and subsequent expression of the fusion genes in a biological expression system.

6. Fusion peptides according to claim 5, characterised in that one or more peptides according to anyone of the SEQ ID NO:1 through SEQ ID NO. 25 are used as linker or as part thereof in order to link the variable domains of a single chain Fv fragment.

7. Fusion peptides according to claim 5, characterised in that one ore more peptides according to anyone of the SEQ ID NO:1 through SEQ ID NO: 25 substitute one or more parts of the peptide sequence of a monoclonal antibody.

8. Fusion peptides according to claims 5 and 7, characterised in that one ore more peptides according to one of the SEQ ID NO: 1 through SEQ ID NO: 25 are expressed as part of one or more hypervariable regions of a monoclonal antibody.

9. Fusion peptides according to anyone of claims 5, 7 or 8, characterised in that they are either expressed, or chemically or enzymatically synthesized as part of a single chain Fv fragment or as part of a Fab fragment.

10. Fusion peptides according to claim 5, characterised in that one or more peptides according to anyone of the SEQ ID NO:1 through SEQ ID NO: 25 substitute one or more parts of the peptide sequence of a viral protein, or are inserted into antigenic sites of a viral protein.

11. Fusion peptides according to claim 10, characterised in that they are part of a virus.

12. Fusion peptides according to claim 10 or 11, characterised in that the viral protein is the hemagglutinin or neuraminidase of influenza virus.

13. Fusion peptides according to claim 10 or 11, characterised in that the viral protein is the surface antigen or the core antigen of hepatitis B virus.

14. Use of peptides as defined in anyone of claims 1 through 5 to select antibodies or antibody fragments binding to HIV-1 *in vitro*.

15. Use of peptides as defined in anyone of claims 1 through 5 in an immunological test in vitro to determine the neutralization titer in complete sera of patients or experimental animals infected with HIV-1, or to determine the status of infection or to make a prognosis on the further progress of infection.

16. Use of peptides as defined in anyone of claims 1 to 5 for the manufacture of antiidiotypic antibodies.

17. A vaccine against HIV-1, characterised in that it comprises at least one peptide and/or fusion peptide as defined in anyone of claims 1 to 13 and/or at least one antiidiotypic antibody obtained according to claim 16.

Claims for the following Contracting State : ES

1. A method for the manufacture of peptides which bind to antibodies, that show neutralizing activity against genetically divergent strains and clinical isolates of HIV-1 and which inhibit the fusion of cells caused by HIV-1, characterized in that oligonucleotides corresponding to one of the amino acid sequences of SEQ ID NO: 1 through SEQ ID NO: 25 are cloned, transformed into and expressed in E.coli, preferably E. coli DH5 α.

2. A method according to Claim 1, characterized in that the peptides are expressed as fusion proteins with glutathion S-transferase (GST), preferably with said oligonucleotides being hybridized and cloned between the Bam HI and Eco RI site of the plasmid pGEX-2T (Pharmacia).

3. A method according to Claim 1 or 2, characterized in that after expression of said amino acid sequences and/or said peptides the E. coli cells are disrupted and said amino acid sequences and/or said peptides are separated from the liquid fraction and purified.

4. A method according to Claim 3, characterized in that disruption of the E. coli cells is achieved by sonication.

5. A method according to Claim 3 or 4, characterized in that the separation and purification of the amino acid sequences and/or peptides is carried out by affinity chromatography, preferably using a glutathion sepharose 4B column, wherefrom the amino acid sequences and/or peptides are eluted, preferably with a solution containing glutathione, NaCl and a buffer.

6. A method according to Claim 5, characterized in that the elution medium is a solution composed of 20 mM glutathione and 120 mM NaCl in 100 mM Tris-HCl, pH 8.0

7. A method for the manufacture of fusion proteins which bind to antibodies that show neutralizing activity against different strains and clinical isolates of HIV-1 and which inhibit the fusion of cells caused by HIV-1, characterized in that

   a) at least one of the amino acid sequences of SEQ ID NO: 1 through SEQ ID NO: 25 is introduced into antigenic sites of the hemagglutinin of influenza A virus by in vitro mutagenesis, thereby leading to chimerical

DNA constructs,
b) said chimerical DNA constructs thereafter being RNP-transfected into influenza HK/WSN virus,

whereby chimerical influenza/HIV viruses are created which exhibit antigenic properties of said fusion proteins, said chimerical influenza/HIV viruses preferably being capable of inducing a neutralizing immune response against genetically divergent HIV-1 strains.

8. A method for the manufacture of fusion proteins which bind to antibodies that show neutralizing activity against different strains and clinical isolates of HIV-1 and which inhibit the fusion of cells caused by HIV-1, characterized in that

a) a single chain Fv construct of a neutralizing anti-HIV-gp120 antibody is prepared,
b) at least one of the amino acid sequences of SEQ ID NO: 1 through SEQ ID NO: 25 is inserted into the linker which connects the variable regions of the heavy and light chain of an immunoglobulin molecule,
c) said fusion proteins are then expressed as part of said immunoglobulin molecule containing the inserted amino acid sequence.

9. A method for the manufacture of fusion proteins which bind to antibodies that show neutralizing activity against different strains and clinical isolates of HIV-1 and which inhibit the fusion of cells caused by HIV-1, characterized in that one or more hypervariable regions or parts thereof of a monoclonal antibody are substituted by at least one of the amino acid sequences of the SEQ ID NO: 1 through SEQ ID NO: 25, by using standard methods.

10. A method according to Claim 9, characterized in that said fusion proteins are expressed as single chain Fv fragments in E.coli, thereafter purified and injected into mice, which thereupon leads to the formation of antiidiotypic antibodies that are capable of inducing a neutralizing immune response against genetically divergent HIV-1 strains.

11. A method for the selection of antibodies and/or antibody fragments binding to HIV-1 *in vitro*, characterized in that at least one of the amino acid sequences of the SEQ ID NO: 1 through SEQ ID NO: 25 is used to bind to said antibodies and/or antibody fragments, whereafter the resulting molecules are separated and purified according to standard techniques.

12. A method for the determination of the status of infection of HIV-1 infected humans and/or animals in vitro, characterized in that at least one of the amino acid sequences of the SEQ ID NO: 1 through SEQ ID NO: 25 is added to a serum of an infected patient and/or experimental animal, whereupon an HIV-1 neutralization titer is determined following state of the art methods.

13. Use of peptides or fusion proteins manufactured according to anyone of Claims 1 to 10 to select antibodies and/or antibody fragments binding to HIV-1 *in vitro* and/or to determine neutralization titers in sera of humans or animals.

14. Use of peptides or fusion proteins manufactured according to anyone of Claims 1 to 10 for the manufacture of a vaccine to elicit HIV-1 neutralizing antibodies in humans.

15. Use according to claim 14 to prevent progression of HIV-1 infection to AIDS

16. Use of peptides or fusion proteins manufactured according to anyone of claims 1 to 10 for the manufacture of antiidiotypic antibodies.

17. Peptides binding to antibodies that show neutralizing activity against different strains and clinical isolates of HIV-1 and that inhibit the fusion of cells caused by HIV-1, characterised in that said peptides are composed according to anyone of the SEQ ID NO: 1 through SEQ ID NO: 25.

18. Peptides according to claim 17, characterised in that they are genetically encoded by the nucleotide sequence according to anyone of the SEQ ID NO: 26 through SEQ ID NO: 50 or by sequences that are deduced from SEQ ID NO: 26 through SEQ ID NO: 50 by degeneration.

19. Peptides according to claim 17 or 18, which upon injection into a mammal either alone or in combination with an adjuvant cause an immune response that leads to the generation of HIV-1 neutralizing antibodies.

20. Peptides according to anyone of claims 17 to 19 in combination with an adjuvant, wherein the adjuvant is a substance to which said peptides are bound through chemical interaction.

21. Peptides according to claim 20 in the form of fusion peptides, characterised in that a protein or part of a protein is used as the adjuvant, to which said peptides are bound by fusion of the respective nucleotide sequences and subsequent expression of the fusion genes in a biological expression system.

22. Fusion peptides according to claim 21, characterised in that one or more peptides according to anyone of the SEQ ID NO: through SEQ ID NO: 25 are used as linker or as part thereof in order to link the variable domains of a single chain Fv fragment.

23. Fusion peptides according to claim 21, characterised in that one ore more peptides according to anyone of the SEQ ID NO: 1 through SEQ ID NO: 25 substitute one or more parts of the peptide sequence of a monoclonal antibody.

24. Fusion peptides according to claims 21 and 23, characterised in that one ore more peptides according to one of the SEQ ID NO: 1 through SEQ ID NO: 25 are expressed as part of one or more hypervariable regions of a monoclonal antibody.

25. Fusion peptides according to anyone of claims 21, 23 or 24, characterised in that they are either expressed, or chemically or enzymatically synthesized as part of a single chain Fv fragment or as part of a Fab fragment.

26. Fusion peptides according to claim 21, characterised in that one or more peptides according to anyone of the SEQ ID NO: through SEQ ID NO: 25 substitute one or more parts of the peptide sequence of a viral protein, or are inserted into antigenic sites of a viral protein.

27. Fusion peptides according to claim 26, characterised in that they are part of a virus.

28. Fusion peptides according to claim 26 or 27, characterised in that the viral protein is the hemagglutinin or neuraminidase of influenza virus.

29. Fusion peptides according to claim 26 or 27, characterised in that the viral protein is the surface antigen or the core antigen of hepatitis B virus.

30. Use of peptides as defined in anyone of claims 17 through 21 to select antibodies or antibody fragments binding to HIV-1 *in vitro*.

31. Use of peptides as defined in anyone of claims 17 through 21 in an immunological test in vitro to determine the neutralization titer in complete sera of patients or experimental animals infected with HIV-1, or to determine the status of infection or to make a prognosis on the further progress of infection.

32. Use of peptides as defined in anyone of claims 17 to 21 for the manufacture of antiidiotypic antibodies.

33. A vaccine against HIV-1, characterised in that it comprises at least one peptide and/or fusion peptide as defined in anyone of claims 17 to 29 and/or at least one antiidiotypic antibody obtained according to claim 16.

Patentansprüche

Patentansprüche für folgende Vertragsstaaten : BE, CH, DE, DK, FR, GB, IE, IT, LI, NL, PT, SE

1. Peptide, welche mit Antikörpern eine Bindung eingehen, die eine neutralisierende Aktivität gegenüber verschiedenen Stämmen und klinischen Isolaten von HIV-1 zeigen, und welche die durch HIV-1 verursachte Zellfusion hemmen, dadurch gekennzeichnet, daß die Peptide gemäß einer der SEQ ID NO: 1 bis SEQ ID NO: 25 zusammengesetzt sind.

2. Peptide nach Anspruch 1, dadurch gekennzeichnet, daß sie genetisch durch die Nukleotidsequenz gemäß einer

der SEQ ID NO: 26 bis SEQ ID NO: 50 kodiert ist oder durch Sequenzen, welche durch Degeneration aus SEQ ID NO: 26 bis SEQ ID NO: 50 abgeleitet sind.

3. Peptide nach Anspruch 1 oder 2, welche bei Injektion in ein Säugetier, entweder alleine oder in Kombination mit einem Adjuvans, eine Immunreaktion verursachen, welche zur Erzeugung von HIV-1 neutralisierenden Antikörpern führt.

4. Peptide nach einem der Ansprüche 1 bis 3, in Kombination mit einem Adjuvans, wobei das Adjuvans eine Substanz ist, an welche die Peptide durch chemische Wechselwirkung gebunden sind.

5. Peptide nach Anspruch 4, in Form von Fusionspeptiden, dadurch gekennzeichnet, daß ein Protein oder ein Teil eines Proteins als Adjuvans benützt wird, an welches die Peptide durch Fusion der jeweiligen Nukleotidsequenzen und nachfolgende Expression der Fusionsgene in einem biologischen Expressionssystem gebunden sind.

6. Fusions peptide nach Anspruch 5, dadurch gekennzeichnet, daß ein oder mehrere Peptide gemäß einer der SEQ ID NO: 1 bis SEQ ID NO: 25 als Bindeglied oder als dessen Teil benutzt werden, um die variablen Domänen eines Fv-Fragmentes mit einziger Kette zu verbinden.

7. Fusionspeptide nach Anspruch 5, dadurch gekennzeichnet, daß ein oder mehrere Peptide gemäß einer der SEQ ID NO: 1 bis SEQ ID NO: 25 einen oder mehrere Teile der Peptidsequenz eines monoklonalen Antikörpers substituieren.

8. Fusionspeptide nach den Ansprüchen 5 und 7, dadurch gekennzeichnet, daß ein oder mehrere Peptide gemäß einer der SEQ ID NO: 1 bis SEQ ID NO: 25 als Teil einer oder mehrerer hypervariabler Regionen eines monoklonalen Antikörpers ausgedrückt sind.

9. Fusionspeptide nach einem der Ansprüche 5, 7 oder 8, dadurch gekennzeichnet, daß sie entweder als Teil eines Fv-Fragmentes mit einziger Kette oder als Teil eines Fab-Fragmentes ausgedrückt oder chemisch oder enzymatisch synthetisiert sind.

10. Fusionspeptide nach Anspruch 5, dadurch gekennzeichnet, daß ein oder mehrere Peptide gemäß einer der SEQ ID NO: 1 bis SEQ ID NO: 25 einen oder mehrere Teile der Peptidsequenz eines viralen Proteins substituieren oder in antigene Stellen eines viralen Proteins eingesetzt sind.

11. Fusionspeptide nach Anspruch 10, dadurch gekennzeichnet, daß sie Teil eines Virus sind.

12. Fusionspeptide nach den Ansprüchen 10 oder 11, dadurch gekennzeichnet, daß das virale Protein das Hämagglutinin oder die Neuraminidase eines Grippevirus ist.

13. Fusionspeptide nach den Ansprüchen 10 oder 11, dadurch gekennzeichnet, daß das virale Protein das Oberflächenantigen oder das Kernantigen eines Hepatitis-B-Virus ist.

14. Verwendung von Peptiden nach einem der Ansprüche 1 bis 5, zum Selektieren von mit HIV-1 *in vitro* eine Bindung eingehenden Antikörpern oder Antikörperfragmenten.

15. Verwendung von Peptiden nach einem der Ansprüche 1 bis 5, in einem immunologischen Test *in vitro*, um den Neutralisationstiter in den vollständigen Sera von Patienten oder von mit HIV-1 infizierten Versuchstieren zu bestimmen oder den Status einer Infektion zu bestimmen oder eine Prognose über den weiteren Fortschritt einer Infektion zu stellen.

16. Verwendung von Peptiden nach einem der Ansprüche 1 bis 5, zur Herstellung von antiidiotypischen Antikörpern.

17. Vaccin gegen HIV-1, dadurch gekennzeichnet, daß es wenigstens ein Peptid und/oder Fusionspeptid nach einem der Ansprüche 1 bis 13 und/oder mindestens einen nach Anspruch 16 erhaltenen antiidiotypischen Antikörper aufweist.

Patentansprüche für folgenden Vertragsstaat : ES

1. Verfahren zur Herstellung von Peptiden, welche mit Antikörpern eine Bindung eingehen, die eine neutralisierende Aktivität gegenüber genetisch divergierenden Stämmen und klinischen Isolaten von HIV-1 zeigen, und welche die durch HIV-1 verursachte Zellfusion hemmen, dadurch gekennzeichnet, daß einer der Aminosäuresequenzen SEQ ID NO: 1 bis SEQ ID NO: 25 entsprechende Oligonukleotide geklont und in E. coli, vorzugsweise E. coli DH5 α, transformiert und ausgedrückt werden.

2. Verfahren nach Anspruch 1, dadurch gekennzeichnet, daß die Peptide als Fusionsproteine mit Glutathion-S-Transferase (GST) ausgedrückt werden, wobei die Oligonukleotide vorzugsweise hybridisiert und zwischen der Bam H1- und der Eco RI-Stelle des Plasmids pGEX-2T (Pharmacia) geklont werden.

3. Verfahren nach Anspruch 1 oder 2, dadurch gekennzeichnet, daß die E.-coli-Zellen nach dem Ausdrücken der Aminosäuresequenzen und/oder Peptide aufgebrochen und die Aminosauresequenzen und/oder Peptide von der flüssigen Fraktion abgetrennt und gereinigt werden.

4. Verfahren nach Anspruch 3, dadurch gekennzeichnet, daß das Aufbrechen der E.-coli-Zellen durch Beschallung erreicht wird.

5. Verfahren nach Anspruch 3 oder 4, dadurch gekennzeichnet, daß das Abtrennen und die Reinigung der Aminosäuresequenzen und/oder Peptide durch Affinitätschromatographie, vorzugsweise unter Verwendung einer Glutathion-Sepharose-4B-Kolonne, durchgeführt wird, wovon die Aminosäuresequenzen und/oder Peptide, vorzugsweise mit einer Glutathion, NaCl und einen Puffer enthaltenden Lösung, eluiert werden.

6. Verfahren nach Anspruch 5, dadurch gekennzeichnet, daß das Elutierungsmedium eine aus 20 mM Glutathion und 120 mM NaCl in 100 mM Tris-HCl zusammengesetzte Lösung, pH-Wert 8,0, ist.

7. Verfahren zur Herstellung von Fusionspeptiden, welche mit Antikörpern eine Bindung eingehen, die eine neutralisierende Aktivität gegenüber verschiedenen Stämmen und klinischen Isolaten von HIV-1 zeigen, und welche die durch HIV-1 verursachte Zellfusion hemmen, dadurch gekennzeichnet, daß

   a) wenigstens eine der Aminosauresequenzen SEQ ID NO: 1 bis SEQ ID NO: 25 durch Mutagenese *in vitro* an Antigenstellen des Hämagglutinin des Grippe-A-Virus eingeführt wird und dadurch zu schmimärischen DNA-Konstruktionen führt,
   b) wonach die schmimärischen DNA-Konstruktionen in ein Grippe-HK/WSN-Virus RNP-transfiziert werden,

   wodurch schimärische Grippe/HIV-Viren geschaffen werden, die antigene Eigenschaften der Fusionsproteine aufweisen, wobei die schimärischen Grippe/HIV-Viren vorzugsweise zum Induzieren einer neutralisierenden Immunreaktion gegen genetisch divergierende HIV-1-Stämme fähig sind.

8. Verfahren zur Herstellung von Fusionspeptiden, welche mit Antikörpern eine Bindung eingehen, die eine neutralisierende Aktivität gegenüber verschiedenen Stämmen und klinischen Isolaten von HIV-1 zeigen, und welche die durch HIV-1 verursachte Zellfusion hemmen, dadurch gekennzeichnet, daß

   a) es wird eine Fv-Konstruktion eines neutralisierenden Anti HIV-gp 120-Antikörpers mit einer einzigen Kette hergestellt,
   b) wenigstens eine der Aminosäuresequenzen SEQ ID NO: 1 bis SEQ ID NO: 25 wird in das Bindeglied eingeführt, die die variablen Regionen der schweren und der leichten Kette eines Immunoglobulin-Moleküls verbindet,
   c) die Fusionsproteine werden sodann als Teil das die eingeführte Aminosäuresequenz enthaltenden Immunoglobulin-Moleküls ausgedrückt.

9. Verfahren zur Herstellung von Fusionspeptiden, welche mit Antikörpern eine Bindung eingehen, die eine neutralisierende Aktivität gegenüber verschiedenen Stämmen und klinischen Isolaten von HIV-1 zeigen, und welche die durch HIV-1 verursachte Zellfusion hemmen, dadurch gekennzeichnet, daß ein oder mehrere hypervariable(r) Bereich(e) oder Teile davon eines monoklonalen Antikörpers durch mindestens eine der Aminosäuresequenzen SEQ ID NO: 1 bis SEQ ID NO: 25 unter Anwendung von Standardverfahren substituiert wird (werden).

10. Verfahren nach Anspruch 9, dadurch gekennzeichnet, daß die Fusionsproteine als Fv-Fragmente in E. coli mit einziger Kette ausgedrückt werden, danach gereinigt und in Mäuse injiziert werden, was dabei zur Bildung von antiidiotypischen Antikörpern führt, die dazu fähig sind, eine neutralisierende Immunreaktion gegen genetisch divergierende HIV-1-Stamme zu induzieren.

11. Verfahren zur Auswahl von Antikörpern und/oder Antikörper-Fragmenten, die mit HIV-1 *in vitro* eine Bindung eingehen, dadurch gekennzeichnet, daß mindestens eine der Aminosäuresequenzen SEQ ID NO: 1 bis SEQ ID NO: 25 zum Binden der Antikörper und/oder Antikörper-Fragmente verwendet wird, worauf die sich ergebenden Moleküle nach Standardverfahren abgetrennt und gereinigt werden.

12. Verfahren zum Bestimmen des Infektionsstatus von mit HIV-1 infizierten Menschen und/oder Tieren *in vitro*, dadurch gekennzeichnet, daß mindestens eine der Aminosäuresequenzen SEQ ID NO: 1 bis SEQ ID NO: 25 dem Serum eines infizierten Patienten und/oder Versuchstieres zugefügt wird, worauf ein HIV-I-Neutralisationstiter nach Verfahren des Standes der Technik bestimmt wird.

13. Verwendung von Peptiden oder Fusionspeptiden, die nach einem der Ansprüche 1 bis 10 hergestellt sind, um Antikörper und/oder Antikörper-Fragmente auszuwählen, die mit HIV-1 *in vitro* eine Bindung eingehen und/oder zum Bestimmen von Neutralisationstitern in Sera von Menschen oder Tieren.

14. Verwendung von Peptiden oder Fusionspeptiden, die nach einem der Ansprüche 1 bis 10 hergestellt sind, für die Herstellung eines Vaccins zum Hervorrufen von HIV-1 neutralisierenden Antikörpern bei Menschen.

15. Verwendung nach Anspruch 14, zum Verhindern des Fortschreitens einer HIV-1-Infektion zu AIDS.

16. Verwendung von Peptiden oder Fusionspeptiden, die nach einem der Ansprüche 1 bis 10 hergestellt sind, für die Erzeugung von antiidiotypischen Antikörpern.

17. Peptide, welche mit Antikörpern eine Bindung eingehen, die eine neutralisierende Aktivität gegenüber verschiedenen Stämmen und klinischen Isolaten von HIV-1 zeigen, und welche die durch HIV-1 verursachte Zellfusion hemmen, dadurch gekennzeichnet, daß die Peptide gemäß einer der SEQ ID NO: 1 bis SEQ ID NO: 25 zusammengesetzt sind.

18. Peptide nach Anspruch 17, dadurch gekennzeichnet, daß sie genetisch durch die Nukleotidsequenz gemäß einer der SEQ ID NO: 26 bis SEQ ID NO: 50 kodiert ist oder durch Sequenzen, welche durch Degeneration aus SEQ ID NO: 26 bis SEQ ID NO: 50 abgeleitet sind.

19. Peptide nach Anspruch 17 oder 18, welche bei Injektion in ein Säugetier, entweder alleine oder in Kombination mit einem Adjuvans, eine Immunreaktion verursachen, welche zur Erzeugung von HIV-1 neutralisierenden Antikörpern führt.

20. Peptide nach einem der Ansprüche 17 bis 19, in Kombination mit einem Adjuvans, wobei das Adjuvans eine Substanz ist, an welche die Peptide durch chemische Wechselwirkung gebunden sind.

21. Peptide nach Anspruch 20, in Form von Fusionspeptiden, dadurch gekennzeichnet, daß ein Protein oder ein Teil eines Proteins als Adjuvans benützt wird, an welches die Peptide durch Fusion der jeweiligen Nukleotidsequenzen und nachfolgende Expression der Fusionsgene in einem biologischen Expressionssystem gebunden sind.

22. Fusionspeptide nach Anspruch 21, dadurch gekennzeichnet, daß ein oder mehrere Peptide gemäß einer der SEQ ID NO: 1 bis SEQ ID NO: 25 als Bindeglied oder als dessen Teil benutzt werden, um die variablen Domänen eines Fv-Fragmentes mit einziger Kette zu verbinden.

23. Fusionspeptide nach Anspruch 21, dadurch gekennzeichnet, daß ein oder mehrere Peptide gemäß einer der SEQ ID NO: 1 bis SEQ ID NO: 25 einen oder mehrere Teile der Peptidsequenz eines monoklonalen Antikörpers substituieren.

24. Fusionspeptide nach den Ansprüchen 21 und 23, dadurch gekennzeichnet, daß ein oder mehrere Peptide gemäß einer der SEQ ID NO: 1 bis SEQ ID NO: 25 als Teil einer oder mehrerer hypervariabler Regionen eines monoklonalen Antikörpers ausgedrückt sind.

25. Fusionspeptide nach einem der Ansprüche 21, 23 oder 24, dadurch gekennzeichnet, daß sie entweder als Teil eines Fv-Fragmentes mit einziger Kette oder als Teil eines Fab-Fragmentes ausgedrückt oder chemisch oder enzymatisch synthetisiert sind.

26. Fusionspeptide nach Anspruch 21, dadurch gekennzeichnet, daß ein oder mehrere Peptide gemäß einer der SEQ ID NO: 1 bis SEQ ID NO: 25 einen oder mehrere Teile der Peptidsequenz eines viralen Proteins substituieren oder in antigene Stellen eines viralen Proteins eingesetzt sind.

27. Fusionspeptide nach Anspruch 26, dadurch gekennzeichnet, daß sie Teil eines Virus sind.

28. Fusionspeptide nach den Ansprüchen 26 oder 27, dadurch gekennzeichnet, daß das virale Protein das Hämagglutinin oder die Neuraminidase eines Grippevirus ist.

29. Fusionspeptide nach den Ansprüchen 26 oder 27, dadurch gekennzeichnet, daß das virale Protein das Oberflächenantigen oder das Kernantigen eines Hepatitis-B-Virus ist.

30. Verwendung von Peptiden nach einem der Ansprüche 17 bis 21, zum Selektieren von mit HIV-1 *in vitro* eine Bindung eingehenden Antikörpern oder Antikörperfragmenten.

31. Verwendung von Peptiden nach einem der Ansprüche 17 bis 21, in einem immunologischen Test *in vitro*, um den Neutralisationstiter in den vollständigen Sera von Patienten oder von mit HIV-1 infizierten Versuchstieren zu bestimmen oder den Status einer Infektion zu bestimmen oder eine Prognose über den weiteren Fortschritt einer Infektion zu stellen.

32. Verwendung von Peptiden nach einem der Ansprüche 17 bis 21, zur Herstellung von antiidiotypischen Antikörpern.

33. Vaccin gegen HIV-1, dadurch gekennzeichnet, daß es wenigstens ein Peptid und/oder Fusionspeptid nach einem der Ansprüche 17 bis 29 und/oder mindestens einen nach Anspruch 16 erhaltenen antiidiotypischen Antikörper aufweist.

Revendications

Revendications pour les Etats contractants suivants : BE, CH, DE, DK, FR, GB, IE, IT, LI, NL, PT, SE

1. Peptides se liant à des anticorps qui présentent une activité neutralisante vis-à-vis de différentes souches et isolats cliniques du HIV-1 et qui inhibent la fusion de cellules induite par le HIV-1, caractérisés en ce que lesdits peptides sont composés selon l'une quelconque des Séquences identifiées par les numéros 1 à 25 .

2. Peptides selon la revendication 1, caractérisés en ce qu'ils sont génétiquement encodés par la séquence nucléotidique selon l'une quelconque des Séquences identifiées par les numéros 26 à 50 ou les séquences qui sont déduites des Séquences identifiées par les numéros 26 à 50 par dégénérescence.

3. Peptides selon la revendication 1 ou 2, qui suite à leur injection à un mammifère, soit séparément soit en conjonction avec un adjuvant, induisent une réponse immune qui conduit à la production d'anticorps neutralisants anti-HIV-I.

4. Peptides selon l'une quelconque des revendications 1 à 3 en association avec un adjuvant, dans lesquels l'adjuvant est une substance à laquelle lesdits peptides se trouvent liés par liaison chimique.

5. Peptides selon la revendication 4 sous forme de peptides de fusion, caractérisés en ce qu'une protéine ou une partie d'une protéine est utilisée comme adjuvant, à laquelle lesdits peptides sont liés par fusion des séquences nucléotidiques respectives et expression ultérieure des gènes de fusion dans un système d'expression biologique.

6. Peptides de fusion selon la revendication 5, caractérisés en ce qu'un ou plusieurs peptides selon l'une quelconque des Séquences identifiées par les numéros 1 à 25 sont utilisés comme segment de jonction ou comme une partie de tel segment afin de joindre les domaines variables d'un fragment Fv à chaîne unique.

7. Peptides de fusion selon la revendication 5, caractérisés en ce qu'un ou plusieurs peptides selon l'une quelconque des Séquences identifiées par les numéros 1 à 25 se substituent à une ou plusieurs parties de la séquence peptidique d'un anticorps monoclonal.

8. Peptides de fusion selon les revendications 5 et 7, caractérisés en ce qu'un ou plusieurs peptides selon l'une quelconque des Séquences identifiées par les numéros 1 à 25 sont exprimés sous forme d'une partie d'une ou de plusieurs régions hypervariables d'un anticorps monoclonal.

9. Peptides de fusion selon l'une quelconque des revendications 5, 7 ou 8, caractérisés en ce qu'ils sont soit exprimés, soit synthétisés par voie chimique ou enzymatique sous forme d'une partie d'un fragment Fv à chaîne unique ou d'une partie d'un fragment Fab.

10. Peptides de fusion selon la revendication 5, caractérisés en ce qu'un ou plusieurs peptides selon l'une quelconque des Séquences identifiées par les numéros 1 à 25 se substituent à une ou plusieurs parties de la séquence peptidique d'une protéine virale, ou sont insérés dans des sites antigéniques d'une protéine virale.

11. Peptides de fusion selon la revendication 10, caractérisés en ce qu'ils font partie d'un virus.

12. Peptides de fusion selon la revendication 10 ou 11, caractérisés en ce que la protéine virale est l'hémagglutinine ou la neuraminidase du virus de la grippe.

13. Peptides de fusion selon la revendication 10 ou 11, caractérisés en ce que la protéine virale est l'antigène de surface ou l'antigène nucléocapsidique du virus de l'hépatite B.

14. Utilisation de peptides tels que définis dans l'une quelconque des revendications 1 à 5 pour sélectionner des anticorps ou des fragments d'anticorps se liant au HIV-I *in vitro*.

15. Utilisation de peptides tels que définis dans l'une quelconque des revendications 1 à 5 dans un essai immunologique *in vitro* pour déterminer le titre d'anticorps neutralisants sur sérum total de patients ou d'animaux de laboratoire infectés par le HIV-I, ou pour déterminer le stade d'infection ou pour formuler un pronostic sur l'évolution de l'infection.

16. Utilisation de peptides tels que définis dans l'une quelconque des revendications 1 à 5 pour l'obtention d'anticorps anti-idiotypiques.

17. Vaccin contre le HIV-I, caractérisé en ce qu'il comprend au moins un peptide et/ou peptide de fusion tel que défini dans l'une quelconque des revendications 1 à 13 et/ou au moins un anticorps anti-idiotypique obtenu selon la revendication 16.

Revendications pour l'Etat contractant suivant : ES

1. Procédé de production de peptides se liant à des anticorps, qui présentent une activité neutralisante vis-à-vis de souches et d'isolats cliniques génétiquement divergents du HIV-I, et qui inhibent la fusion de cellules induite par le HIV-I, caractérisé en ce que les oligonucléotides correspondants à l'une des séquences d'acides aminés encodées par les séquences identifiées par les numéros 1 à 25 sont clonés, transformés et exprimés dans E. coli, de préférence dans E. coli DH5α.

2. Procédé selon la revendication 1, caractérisé en ce que les peptides sont exprimés comme protéines de fusion à la glutathione S-transférase (GST), de préférence en hybridant et en clonant lesdits oligonucléotides entre le site Bam HI et Eco RI du plasmide pGEX-2T (Pharmacia).

3. Procédé selon la revendication 1 ou 2, caractérisé en ce que suite à l'expression desdites séquences d'acides aminés et/ou desdits peptides, il y a rupture des cellules d'E. coli et séparation et purification desdites séquences d'acides aminés et/ou desdits peptides de la fraction liquide.

4. Procédé selon la revendication 3, caractérisé en ce que la rupture des cellules d'E. coli s'obtient par sonication.

5. Procédé selon la revendication 3 ou 4, caractérisé en ce que la séparation et la purification des séquences d'acides aminés et/ou des peptides s'effectuent par chromatographie d'affinité, de préférence à l'aide d'une colonne de type glutathione sepharose 4B, à partir de laquelle les séquences d'acides aminés et/ou les peptides sont élués, de préférence avec une solution contenant du glutathione, NaCl et un tampon.

6. Procédé selon la revendication 5, caractérisé en ce que le milieu d'élution est une solution composée de glutathione 20 mM et de NaCl 120 mM dans du Tris 100 mM-HCl, pH 8,0.

7. Procédé d'obtention de protéines de fusion se liant à des anticorps qui présentent une activité neutralisante vis-à-vis de différentes souches et isolats cliniques du HIV-I et qui inhibent la fusion de cellules induite par le HIV-I, caractérisé en ce que

a) au moins l'une des séquences d'acides aminés correspondant aux séquences identifiées par les numéros 1 à 25 est introduite dans des sites antigéniques de l'hémagglutinine du virus de la grippe A par mutagenèse *in vitro*, de manière à obtenir des constructions d'ADN chimères,
b) lesdites constructions d'ADN chimères sont ensuite transfectées par RNP dans des virus de la grippe HK/WSN,

de sorte qu'il y a production de virus de la grippe/HIV chimères qui présentent des propriétés antigéniques desdites protéines de fusion, lesdits virus de la grippe/HIV chimères étant de préférence capables d'induire une réponse immune neutralisante vis-à-vis de souches de HIV-1 génétiquement divergentes.

8. Procédé d'obtention de protéines de fusion se liant à des anticorps qui présentent une activité neutralisante vis-à-vis de différentes souches et isolats cliniques du HIV-I et qui inhibent la fusion de cellules induite par le HIV-1, caractérisé en ce que

a) une construction Fv à chaîne unique d'un anticorps anti-gp120 de HIV est préparée,
b) au moins l'une des séquences d'acides aminés correspondant aux Séquences identifiées par les numéros 1 à 25 est insérée dans le segment de jonction qui relie les régions variables de la chaîne lourde et légère d'une molécule d'immunoglobuline,
c) lesdites protéines de fusion sont ensuite exprimées sous forme d'une partie de ladite molécule d'immunoglobuline contenant la séquence d'acides aminés insérée.

9. Procédé d'obtention de protéines de fusion se liant à des anticorps qui présentent une activité neutralisante vis-à-vis de différentes souches et isolats cliniques du HIV-1 et qui inhibent la fusion de cellules induite par le HIV-1, caractérisé en ce qu'une ou plusieurs régions hypervariables ou parties de telles régions d'un anticorps monoclonal sont substituées par au moins l'une des séquences d'acides aminés correspondant aux Séquence identifiées par les numéros 1 à 25, en utilisant des techniques courantes.

10. Procédé selon la revendication 9, caractérisé en ce que lesdites protéines de fusion sont exprimées sous forme de fragments Fv à chaîne unique dans E. coli, puis purifiées et injectées dans des souris, ce qui aboutit ensuite à la formation d'anticorps anti-idiotypiques capables d'induire une réponse immune neutralisante vis-à-vis de souches du HIV-I génétiquement divergentes.

11. Procédé de sélection d'anticorps et/ou de fragments d'anticorps se liant au HIV-1 *in vitro*, caractérisé en ce qu'au moins l'une des séquences d'acides aminés correspondant aux Séquences identifiées par les numéros 1 à 25 est utilisée pour fixer lesdits anticorps et/ou fragments d'anticorps, à la suite de quoi les molécules produites sont séparées et purifiées selon des techniques standards.

12. Procédé de détermination *in vitro* du stade d'infection chez des êtres humains et/ou des animaux infectés par le HIV-1, caractérisé en ce qu'au moins l'une des séquences d'acides aminés correspondant aux Séquences identifiées par les numéros 1 à 25 est ajoutée à un sérum d'un malade infecté et/ou d'un animal de laboratoire, à la suite de quoi le titre d'anticorps neutralisants anti-HIV-1 est déterminé suivant des techniques conventionnelles.

13. Utilisation de peptides ou de protéines de fusion obtenus selon l'une quelconque des revendications 1 à 10 pour sélectionner des anticorps et/ou des fragments d'anticorps se liant au HIV-1 *in vitro* et/ou pour déterminer les titres d'anticorps neutralisants dans les sérums humains ou animaux.

14. Utilisation de peptides ou de protéines de fusion obtenus selon l'une quelconque des revendications 1 à 10 dans l'obtention d'un vaccin destiné à induire la production d'anticorps neutralisants anti-HIV-1.

15. Utilisation selon la revendication 14 pour empêcher l'évolution de l'infection par le HIV-1 vers le SIDA.

16. Utilisation de peptides ou de protéines de fusion obtenues selon l'une quelconque des revendications 1 à 10 pour l'obtention d'anticorps anti-idiotypiques.

17. Peptides se liant à des anticorps qui présentent une activité neutralisante vis-à-vis de différentes souches et isolats cliniques du HIV-1 et qui inhibent la fusion de cellules induite par le HIV-I, caractérisés en ce que lesdits peptides sont composés selon l'une quelconque des Séquences identifiées par les numéros 1 à 25 .

18. Peptides selon la revendication 17, caractérisés en ce qu'ils sont génétiquement encodés par la séquence nucléotidique selon l'une quelconque des séquences identifiées par les numéros 26 à 50 ou des séquences qui sont déduites des Séquences identifiées par les numéros 26 à 50 par dégénérescence.

19. Peptides selon la revendication 17 ou 18, qui suite à leur injection dans un mammifère, soit séparément soit en conjonction avec un adjuvant, induisent une réponse immune qui conduit à la production d'anticorps neutralisants anti-HIV-I.

20. Peptides selon l'une quelconque des revendications 17 à 19 en association avec un adjuvant, dans lesquels l'adjuvant est une substance à laquelle lesdits peptides se trouvent liés par liaison chimique.

21. Peptides selon la revendication 20 sous forme de peptides de fusion, caractérisés en ce qu'une protéine ou une partie de protéine est utilisée comme adjuvant, à laquelle lesdits peptides sont liés par fusion des séquences nucléotidiques respectives et expression ultérieure des gènes de fusion dans un système d'expression biologique.

22. Peptides de fusion selon la revendication 21, caractérisés en ce qu'un ou plusieurs peptides selon l'une quelconque des Séquences identifiées par les numéros 1 à 25 sont utilisés comme segments de jonction ou comme une partie de tel segment afin de joindre les domaines variables d'un fragment Fv à chaîne unique.

23. Peptides de fusion selon la revendication 21, caractérisés en ce qu'un ou plusieurs peptides selon l'une quelconque des Séquences identifiées par les numéros 1 à 25 se substituent à une ou plusieurs parties de la séquence peptidique d'un anticorps monoclonal.

24. Peptides de fusion selon les revendications 21 et 23, caractérisés en ce qu'un ou plusieurs peptides selon l'une quelconque des Séquences identifiées par les numéros 1 à 25 sont exprimés sous forme d'une partie d'une ou de plusieurs régions hypervariables d'un anticorps monoclonal.

25. Peptides de fusion selon l'une quelconque des revendications 21, 23 ou 24, caractérisés en ce qu'ils sont soit exprimés, soit synthétisés par voie chimique ou enzymatique sous forme d'une partie d'un fragment Fv à chaîne unique ou d'une partie d'un fragment Fab.

26. Peptides de fusion selon la revendication 21, caractérisés en ce qu'un ou plusieurs peptides selon l'une quelconque des Séquences identifiées par les numéros 1 à 25 se substituent à une ou plusieurs parties de la séquences peptidique d'une protéine virale, ou sont insérés dans des sites antigéniques d'une protéine virale.

27. Peptides de fusion selon la revendication 26, caractérisés en ce qu'ils font partie d'un virus.

28. Peptides de fusion selon la revendication 26 ou 27, caractérisés en ce que la protéine virale est l'hémagglutinine ou la neuraminidase du virus de la grippe.

29. Peptides de fusion selon la revendication 26 ou 27, caractérisés en ce que la protéine virale est l'antigène de surface ou l'antigène nucléocapsidique du virus de l'hépatite B.

30. Utilisation de peptides tels que définis dans l'une quelconque des revendications 17 à 21 pour sélectionner des anticorps ou des fragments d'anticorps se liant au HIV-I *in vitro*.

31. Utilisation de peptides tels que définis dans l'une quelconque des revendications 17 à 21 dans un essai immunologique *in vitro* pour déterminer le titre d'anticorps neutralisants sur sérum total de patients ou d'animaux de laboratoire infectés par le HIV-I, ou pour déterminer le stade d'infection ou pour formuler un pronostic sur l'évolution de l'infection.

32. Utilisation de peptides tels que définis dans l'une quelconque des revendications 17 à 21 pour l'obtention d'anticorps anti-idiotypiques.

33. Vaccin contre le HIV-I, caractérisé en ce qu'il comprend au moins un peptide et/ou peptide de fusion tel que défini dans l'une quelconque des revendications 17 à 29 et/ou au moins un anticorps anti-idiotypique obtenu selon la revendication 16.

Abb.1a

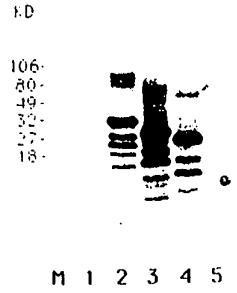
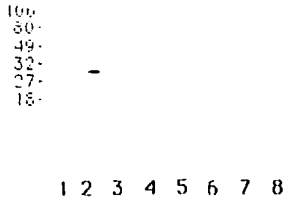
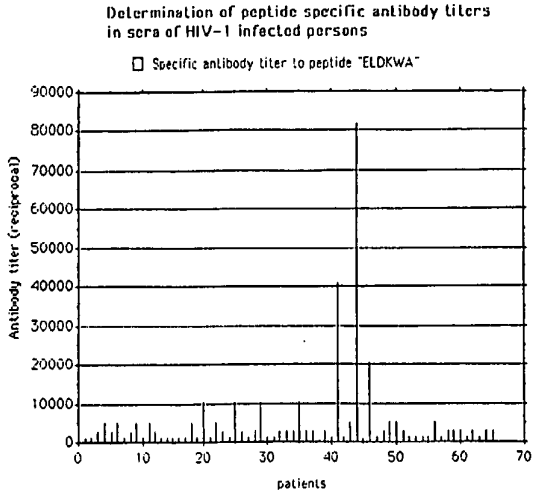


Abb.1b

Abb.1c





The antibody titres were determined by ELISA. The peptid was used in form of a fusionpeptide (in combination with glutathione-S-transferase). The fusionpeptide was coated to 96 microtitre plates 100µl/well (2,5µg/ml) and incubated over night at 4°C. After washing three times with washing-buffer HIV-1 postitive sera were diluted 2^n fold (1:40-1:81920) in dilution-buffer and aliquotes were transfered to the test-plate (100µl/well) and incubated for 1h at RT. Then the plates were washed again three times with washing-buffer. As a second antibody goat anti human γ-chain, conjugated with horse radish peroxidase, was used (diluted 1:1000, 100µl/well). After 1h incubation at RT the plates were washed three times with washing-buffer. Then the plates were stained using o-phenylene-diamine-dihydrochloride as substrate. The reaction was stopped with 2,5 M H_2SO_4 and the plates were measured (measure wavelength 492 nm, reference wavelength 620 nm) and evaluated.

Cutoff = the mean value (4-fold) of a HIV-1 negative serum (1:40) + 3 fold standard deviation. The donors of serum number 20, 25, 29, 35, 41, 44, 46 are HIV-1 positive for at least five years and still asymptomatic.

Figure 2: Graphic of the specific antibody titres to the peptide with the aminoacid sequence "ELDKWA" of 65 sera from HIV-1 positive donors

Influenza/HIV inhibition of HIV-1 IIIB neutralization

monoklonal antibodies:	residual IIIB neutralizationstiter in % after inkubation with:		
	Mock	Influenza WSN	Influenza/HIV
2F5	100%	100%	10%
2G12	100%	100%	100%

Figure 3a: Influenza/HIV inhibition of HIV-1 neutralization. Results are expressed as reciprocal of the serum dilution giving > 90% reduction in HIV titer following preincubation of the mABs 2F5 and 2G12 with culture medium (Mock), influenza WSN or influenza/HIV. 2F5 is the monoclonal antibody specific for the different epitope on gp160. Residual HIV-neutralizing activity was determined by incubating dilutions of the antibody/virus mixture with 10^3 infectious units ($TCID_{50}$) of HIV-1 IIIb for 1h at 37°C. Aliquots (100µl) of medium containing 10^4 C8166 cells were added and the presence of syncytia recorded after 48h as an indication of HIV infection.

Immunogen:	Antibodytiter:
K1	< 10
K2	< 10
K3	< 10
F1	1600
F2	6400
F3	800
SM1	3200
SM2	400
SM3	800
V1	3200
V2	3200
V3	6400
rA1	< 10
rA2	400
rA3	800

Figure 3b: Antibodytiter. Three Balb/c mice each were immunized with either 100µg GST (K), 100µg Fusion protein (F), 100µg of the "immunological supermolecule", 100µg of the recombinant antiidiotypic antibody (rA) or 4.0 $\log_{10}TCID_{50}$ of the recombinant influenza/HIV virus (V) and were boostered after 2 and 4 weeks. One week after the last immunization ELISA antibody were determined. Results are given as reciprocal values that gave significant positive values. The cutoff was the double value of a normal mouse serum. Recombinant gp41 was used as an antigen.

Reciprocal neutralization titers of HIV-1 isolates

Antiserum	IIIB	RF	MN
P1	40	40	40
P2	80	40	40
P3	40	20	20
V1	40	80	40
V2	20	40	<10
V3	160	80	80
SM1	20	40	40
SM2	40	80	80
SM3	<10	<10	<10
rA1	40	40	40
rA2	40	80	20
rA3	80	40	40

Figure 3c: Neutralization of HIV-1 infection. Neutralizationtiters were determined by incubating 10µl of heat inactivated antuserum with 40µl virus supernatant containing 10^3 infectious units of HIV-1 at 37°C for 1h. Residual HIV-1 infectivity was measured as described in fig. 3a. Abbreviations: P ... fusionpeptide. V ... chimeric influenza/HIV virus. SM ... "immunological supermolecule". rA ... recombinant antibody. Reciprocal neutralization titers of all controls were lower than 10.

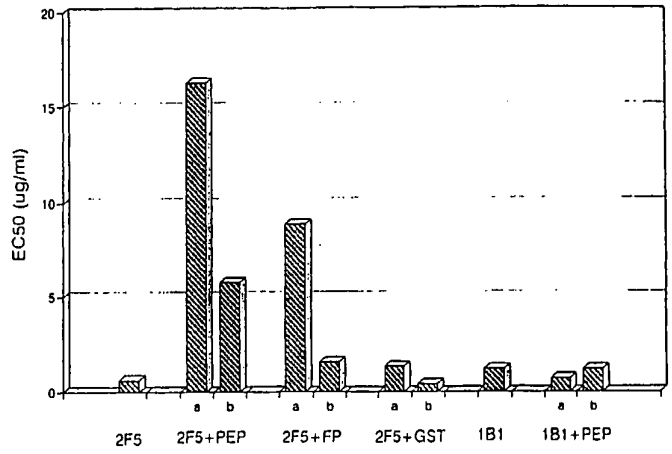


Fig. 4: Inhibition of neutralization by peptides. Synthetic peptide (PEP), fusion peptide (FP), and glutathion-S-transferase (GST) were preincubated with humAb 2F5 or 1B1 for 1 h at 37 °C, and then a syncytia inhibition assay was performed.
Antibodies were diluted in 2-fold steps starting with 5 µg/well.
PEP, synthetic peptide ELDKWA (peptide corresponding to SEQ ID NO: 1): a 25 µg, b 5 µg per well;
FP, fusion peptide ELDKWA with GST: a 25 µg, b 5 µg per well;
GST, glutathion-S-transferase: a 25 µg, b 5 µg per well;
1B1, neutralizing anti gp120 humAb